Abstract

Distribution Patterns of Juvenile Spotted Seatrout (*Cynoscion nebulosus*) and Red Drum (*Sciaenops ocellatus*) along Shallow Beach Habitats in Pamlico River, North Carolina

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The association of juvenile spotted seatrout (*Cynoscion nebulosus)* and red drum (*Sciaenops ocellatus)* with Submerged Aquatic Vegetation (SAV) is well documented. However, their association with other estuarine habitats including shallow (non-vegetated) sandy areas is not well understood. The goal of this project was to evaluate habitat use and distribution of juvenile spotted seatrout and red drum along shallow habitats in Pamlico River, North Carolina. The specific objectives were: 1) to evaluate the spatiotemporal patterns of juvenile spotted seatrout and red drum distribution; 2) to determine the effect of habitat type (SAV, sand, and detritus) on growth and mortality; 3) to determine the accuracy and precision in estimating fish age from otoliths with two methods: polishing and oil immersion; and 4) to distinguish how fish community structure (intraspecific and interspecific networks) may affect the presence of juvenile spotted seatrout and red drum distribution in the fish community. Pamlico River was divided into three 21.6-km strata from Fork Point Island westward, to the mouth of the Pungo

River. The three areas were identified as West, Central, and East and each contained six fixed stations. Juvenile spotted seatrout and red drum were collected twice a month with an 18-m beach seine from August through November 2009 and 2010. Three substrate samples at each site were also collected once during the second sampling season. All fish were weighed (nearest 0.01 mg), measured (TL, SL in mm). Size (TL) ranged from 30 to 160 mm TL for spotted seatrout and from 15 to 65 mm TL for red drum. The West area of Pamlico River had the highest abundance of juvenile spotted seatrout and the Central had the highest abundance of juvenile red drum. Juvenile spotted seatrout hatch dates were most frequent in June, while juvenile red drum were most frequent during August. Red drum were mostly associated with detritus (52%) compared to sand (20%) or SAV (28%), whereas spotted seatrout were primarily associated with SAV (57%). Furthermore, instantaneous growth of spotted seatrout and red drum did not differ among habitats. Results of this study show how a euryhaline environment and habitat type could potentially influence fish distribution patterns. Results herein will support the development and updating of a fishery management plan for spotted seatrout and red drum in North Carolina.

DISTRIBUTION PATTERNS OF JUVENILE SPOTTED SEATROUT (*CYNOSCION NEBULOSUS*) AND RED DRUM (*SCIAENOPS OCELLATUS*) ALONG SHALLOW BEACH HABITATS IN PAMLICO RIVER, NORTH CAROLINA

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INTRODUCTION

Spotted seatrout (*Cynoscion nebulosus*) and red drum (*Sciaenops ocellatus)* are important recreational and commercial fish species found along the east coast of the U.S. from Virginia to Florida and the Gulf Coast (Peebles and Tolley 1988). Schramm et al. (1991) conducted a survey on the number of annual fishing tournaments and showed that out of 582 single-species fishing events, spotted seatrout accounted for 2.7% and red drum accounted for 2.3% of the fishing tournaments. North Carolina red drum commercial landings in 2010 were 78.9 tons, with declines in fishing effort and 1991; Spotted seatrout commercial landings in 2010 were 111.7 tons, also having declines since 1991; in fishing as measured by number of vessels and personal has stayed constant over that period (Burgess and Bianchi 2004; Takade and Paramore 2007; Jensen 2009; NCDMF 2010b).

On the U.S. east coast, fishes and estuarine invertebrates normally follow key salinity gradients that produce distinct aquatic communities (Boesch 1977; Weinstein et al. 1980; Thayer et al. 1999). If there is an increase in freshwater flow into a system from upstream, downstream (hypersaline) areas can become diluted, altering the range and distribution of early life fish species. However, if upstream freshwater flows are restricted, hypersaline areas can extend further upstream (Copeland 1966). Spotted seatrout live and reproduce in estuaries and bays, where salinity can vary from brackish to hypersaline (Holt and Holt 2003; Tsuzuki et al. 2003; Luczkovich et al. 2008b). Some of the most important factors affecting the overall functionality of an estuary are the timing and quality of the freshwater inflow. Additionally, these factors can affect the distribution of spotted seatrout, with larger more mature spotted seatrout at mesohaline and polyhaline sites, and younger less developed fish at oligohaline sites (Montagna et al. 2002).

A tagging study by Holt and Holt (2003) concluded that spotted seatrout migration among related bay systems may not existent. In Florida Bay and Ten Thousand Islands, there was very little inter-estuary movement of spotted seatrout; 95% never moved more than 48.3-km from tagging locations, with only 5% collected up to 506-km from tagged locations (Iversen and Tabb 1962).

The Magnuson-Stevens Fishery Management Act and Fish Habitat Conservation Act of 2008 focused on conserving the Nation's habitats (essential fish habitats) for fishes and aquatic communities. These acts helped establish essential fish habitats, which are habitats that are necessary for fish health and well being as they reach maturity. Restoring overfished stocks and eliminating bycatch were also major concerns (Conservation 1996). These Acts set the tone for establishing Fishery Management Plans (FMPs) and other regulations for marine fishery species, which are regulated by U.S. regional management councils (Fluharty 2000).

The importance of seagrass and salt marsh edges as spawning, feeding and nursery areas for a diversity of juvenile fishes are well known (Stunz et al. 2002a; Bloomfield and Gillanders 2005). Moreover, variations in habitats can significantly influence and dramatically affect growth of juvenile fishes (Baltz et al. 1998). Submerged aquatic vegetation (SAV) beds show the potential for being a major driver in overall fish health and well-being in Pamlico River, North Carolina (Fitzgerald 1998). Similar aquatic fauna gain protection in submerged aquatic vegetation, which reduces predation efficiency and increases growth rate (Rozas and Odum 1988).

The accumulation of detritus in sandy habitats can significantly influence fish abundance, diversity and fish feeding health (Adams 1976; Peterson and Turner 1994). Bachelor et al.

(2009) found that age-1 red drum were more abundant in detritus and less abundant in seagrasses. The importance or role of detritus as habitat has been well document in other systems (Haines 1977; Moore et al. 2004). However, in Pamlico River for spotted seatrout and red drum it is not well understood.

The Pamlico River, North Carolina has various habitats for fishes including seagrass beds, detritus, marsh, and soft bottom (sand), and the presence of these habitats influence fish abundances (Nagelkerken 2001; NCDMF 2010a). These habitats may be dominated by one fish species but a variety of other species recruit to these habitats as well (Gray et al. 1998). If fisheries managers are going to use habitats to manage fish stocks, then knowledge of these areas is essential (Levin and Stunz 2005). In addition, growth, loss rate, and abundance comparisons between habitat types will provide better understanding of how fish species relate to specific habitats.

Life History

Spotted seatrout spawning in North Carolina occurs from April to October and peaks in May and June, primarily because of the seasonality of the photoperiod (Brown-Peterson et al. 1988; Perdue et al. 2010). In Pamlico Sound, spawning times are indicated by drumming of spotted seatrout heard from June until August and peaking in July (Luczkovich et al. 2008b). Unlike the majority of other sciaenid species, adult spotted seatrout spawn within estuaries as opposed to the continental shelf (Brown-Peterson 2003; Smith et al. 2008).

Red drum spawning occurs near mouths of bays, passes, and coastal ocean waters (Peters and McMichael 1987; Matlock 1990). A passive acoustics study by Sprague et al. (2000) showed that from August to November red drum, were heard drumming at the mouth of Bay River, North Carolina, possibly indicating when and where spawning occurs in estuarine systems. Moreover, drumming occured from August to September in Pamlico Sound (Luczkovich et al. 2008b). A sound production study on the Texas coast determined that red drum spawning occurs within inshore coastal ocean regions (Holt 2008). Passive acoustics techniques not only identify spawning areas, but help identify habitats used and relative abundance of species as well (Luczkovich et al. 2008a). With the exception of spotted seatrout, silver perch (*Bairdiella chrysoura*), black drum (*Pogonias cromis*), and weakfish (*Cynoscion regalis*), the adults of most sciaenid species in southeastern U.S. estuaries travel offshore to spawn and use estuaries only during larval and juvenile life stages (Currin et al. 1984; Collins et al. 2003).

Growth

During their first several years of life, spotted seatrout grow quickly and sexually mature at a small size (age-0) (Brown-Peterson 2003; Powell 2003). Peebles and Tolley (1988) concluded that spotted seatrout hatch at 1.5 mm and larvae grew about 0.4 mm per day in southwest Florida estuaries, reaching about 5 mm SL in 12 days. Conversely, red drum hatch at 1.7 mm TL and grow more rapidly with an average of 0.6 mm per day, and have been found to settle in estuarine habitats around 6 to 8 mm SL (Scharf 2000; Smith et al. 2001; Stunz et al. 2002b; Lucas and Southgate 2012).

Growth rates are perhaps a good indicator of subtle biotic and abiotic changes within individual estuaries (Murphy and McMichael 2003). Prey abundance, salinity, temperature, water depth, substrate (habitat) and dissolved oxygen can have dramatic effects on growth of spotted seatrout and red drum (Baltz et al. 1998). Effects of salinity on growth rates and growth efficiency can be temperature-dependent, with optimal salinity for growth increasing with the increase in temperature (Lankford and Targett 1994; Scharf 2000). Favorable temperature range of the juvenile spotted seatrout and red drum growth is from 24.5 to 33.0˚C and 12.5 to 32.2˚C, respectively (Baltz et al. 2003). In addition, temperatures below 4˚C are found to be fatal (Moore 1976). Relative abundance of spotted seatrout and Atlantic croaker *(Micropogonias undulatus)* increased with seasonal differences in water temperature and salinity (Ayvazian et al. 1992; Hare and Able 2007; MacRae and Cowan 2010). Bachman and Rand (2008) proposed that sharp sudden salinity changes have adverse effects on survival, growth, and hatching success of many estuarine fish species.

Affect of Community Composition on Two Species

Estuarine fish communities are comprised of many species and their individual responses to environmental gradients (i.e., salinity and temperature) (Boesch 1977; Weinstein et al. 1980). Red drum can survive in a variety of salinity ranges by its adaptation of replacing water that is lost from osmosis and the removal of salt absorbed by seawater (Wurts 1998). Salinity and photoperiod are the primary abiotic variables affecting temporal and spatial changes in fish assemblages (Subrahmanyam and Coultas 1980; Series 1992; de Morais and De Morais 1994; Thayer et al. 1999). Beyond individual response to physiochemical differences, populations also respond to biotic factors that influence community structure such as seagrass intra- and inter-specific competition among fishes (Rooker et al. 1998). Moreover, biotic factors such as SAV beds affect fish community and distribution of a species (Chester and Thayer 1990). Habitats can play a vital role in fish assemblages. For instance, in Barataria Bay Los Angeles, gafftopsail catfish (*Bagre marinus*), leather jacket (*Oligoplites saurus*), and sub-adult red drum were found inhabiting marsh edge habitats. However, Atlantic croaker, Atlantic needlefish (*Strongylura marina*), gizzard shad (*Dorosoma cepedianum*), Gulf menhaden (*Brevoortia patronus*), sea catfish (*Ariopsis felis*), lady fish (*Albula vulpes*), Spanish mackerel (*Scomberomorus maculatus*), spot (*Leiostomus xanthurus*), spotted seatrout, and threadfin shad *(D. petenense*) were present in all habitats (MacRae 2007).

Fish community analyses offer information regarding species co-occurrences that can help in understanding inter-specific species and environmental relationships (Ter Braak 1994). In a study in Matagorda Bay, Texas, seagrass communities were dominated by spotted seatrout during the summer and autumn months along with silver perch, bay anchovy (*Anchoa mitchilli*), tidewater silverside (*Menidia peninsulae*), southern flounder (*Paralichthys lethostigma*), gizzard shad, black drum, and hardhead catfish (*Arius felis*) (Akin et al. 2003). Seasonality and salinity fluctuations influence fish communities; an example is spotted seatrout and silver perch, which have been shown to co-occur in mid-salinity areas (Series 1992; Guisan et al. 1999). Additionally, spotted seatrout and silver perch abundance can be at their highest during spring and summer in response to emergent seagrasses (Baltz et al. 1993).

Habitat

Estuarine-dependent spotted seatrout adults, juveniles and larvae are lifetime inhabitants of estuaries, and as a result exhibit a high degree of plasticity in growth by relying on local seagrass beds for food, growth and shelter (NCDMF 2010a). Chester and Thayer (1990) found juvenile spotted seatrout and gray snapper (*Lutjanus griseus*) in higher numbers in seagrass beds that were higher in fish species diversity and density. For both spotted seatrout and red drum, small fish (median length 1.5 mm) can be associated with un-vegetated areas and large fish (median length 12.2 mm) associated with shoal weed (*Halodule*) (Tolan et al. 1997). In addition, seagrass and marsh edges generally have the most dense abundance of newly settled juvenile red drum (Stunz et al. 2002a). A study by Rooker and Holt (1997) showed significant difference in juvenile red drum abundances between shoal grass (*Halodule wrightii*) and turtle grass

(*Thalassia testudinum*), with shoal grass being more preferred for newly settling red drum than turtle grass. Red drum in aquatic systems with sparse seagrass beds have been shown to seek oyster reefs and salt marshes, which were the next most complex habitats within the system (Stunz and Minello 2001).

The abundance and distribution of fishes can be strongly related to sediment texture and grain size (McConnaughey and Smith 2000; Phelan et al. 2001). Effects of finely grained well-mixed sediment may also have positive influences on the abundance of juvenile fish in North Wales, UK (Rogers 1992). Likewise, distribution patterns of bluefish (*Pomatomus saltatrix*) in Sandy Hook Bay-Navesink River were related to sediment grain size (flow within the system) (Scharf et al. 2004). Water flow (velocity) may have a significant impact on aquatic biomass in an aquatic system, with higher flows resulting in larger grain size and less nutrient-enriched areas (Chambers et al. 1991).

The purpose of my study was to determine spatial and temporal distribution patterns of juvenile spotted seatrout and red drum in Pamlico River. Specific objectives were: 1) to evaluate the spatio-temporal patterns in juvenile spotted seatrout and red drum distribution and growth in relation to habitat type (SAV, sand, and detritus) in Pamlico River; 2) to determine the effect of habitat type on growth and mortality; 3) to determine the accuracy and precision in estimating otolith ages between polishing and oil immersion; and 4) to distinguish how fish community structure may affect the presence of juvenile spotted seatrout and red drum distribution and abundance.

METHODS

Study Area

Pamlico River is a wind driven system, located in eastern North Carolina. The origin of the river is north of Durham in the Piedmont region of the state where the river is called the Tar River. It meanders southeast until it reaches Washington, NC, where the name changes to Pamlico River (Riggs 1984). Pamlico River is an east flowing river extending roughly 65 km from its headwaters of Washington to where it empties into Pamlico Sound, North Carolina. With an average depth of 4-5 m and about 65 km long, the Pamlico River widens gradually from 0.5 km at Washington to 6.5 km at the mouth of the river (Xu et al. 2008). Pamlico River estuary is a shallow system; the gravitational and salinity stratification likely differs from other deeper systems such as Hudson, James, York, Rappahannock, and Cape Fear River on the east coast of the United States (Xu et al. 2008). Pamlico River is one of the main freshwater tributaries to Pamlico Sound, along with the Neuse River to the south (Copeland et al. 1984). Since there is no unambiguous connection to the open ocean, lunar tidal fluctuations are modest and salinity stratification often occurs in the middle to lower portion of the estuary, but is often distorted by wind events (Giese et al. 1985; Lin et al. 2008). Furthermore, the landscape sounding Pamlico River estuary is comprised of about two-thirds of forested land (Copeland et al. 1984).

Field Collection and Data Processing

Pamlico River was divided into three 21.6 km strata from Fork Point Island extending downstream to the mouth of the Pungo River. Each of the three areas (strata) was identified as West, Central, and East with each containing six fixed sites categorized based the pre-dominant visualized habitat observed (submerged aquatic vegetation (SAV), sand, and detritus). The term detritus was given to identity terrestrial leaves and finely woody debris. West area was the most upstream starting at Fork Point, Central area extended from Sandy Point east toward Bath Creek, and East area was from Hickory Point to Pamlico Point (Fig. 1). Each area was sampled twice each month from October 2009 through November 2009 and from August 2010 through November 2010. All sampling was conducted during the daytime hours.

Fish were collected with a beach bag seine 18.3 m long and1.8 m high constructed of 6.4 mm bar mesh in the body and 3.2-mm bar mesh in the bag. One end of the net was held at the shoreline, while the other end was fully extended perpendicular to the shoreline. The offshore end of the net was pulled in a quarter sweep and returned to the shore while the beach end remained stationary, equaling a total area swept of about 255 m^2 (Reynolds et al. 1996). Bag seines are effective collecting gear for spotted seatrout and red drum ~≤ 100 mm (Bacheler et al. 2008; Purtlebaugh and Allen 2010).

Spotted seatrout and red drum were identified, measured (TL and SL in mm), and transferred into labeled bags where they were placed on ice and transported to the laboratory. Once in the laboratory, fish species were measured once more (TL, and SL in mm) and wet weights where taken to the nearest 0.01 mg. Fish specimens were then preserved in labeled jars with 80% ethanol alcohol. Stomachs were removed from the body cavity and stored in individual sample vials filled with a solution of Rose Bengal and 95% ethanol (see diet analysis section). The weight of each individual stomach without food was recorded.

CTD profile data (temperature (°C), dissolved oxygen (mg/L), percent oxygen saturation (mg/L), and salinity (ppt)) were collected with a YSI Professional Plus monitor. Also, pH, air temperature (°C), wind direction and wind speed (m/s) were recorded. To investigate the water quality parameters, an analysis of variance (ANOVA) was used to examine chemical properties of water contrasted among areas and sites throughout the river. Properties that were significant at level alpha 0.05 or less, were examined further with a Tukeys posthoc test to identify which property was generating significance in the ANOVA.

Spotted seatrout and red drum catch per unit effort (CPUE) geometric mean data were skewed (Zero Inflation) and did not meet the assumption of normality, so a non-parametric Kruskal-Wallis ANOVA was used to evaluate CPUE differences in area (West, Central, and East) and habitat (SAV, sand, and detritus). If significant, a Wilcoxon Rank-Sum test was used to identify differences. To examine spotted seatrout and red drum TL, an analysis of variance (ANOVA) was used to examine differences in spotted seatrout and red drum length among areas.

Sediment Analysis

Sediment type (silt, very fine sand, fine sand, medium-dine sand, medium sand, course sand, gravel, and biological material,(Blott and Pye 2001) was determined by collecting sediment cores using a PVC pipe, 0.2 m and 30 mm in diameter. Three 127-mm deep sediment cores were obtained, transferred to labeled bags, and placed on ice for transport back to the laboratory for later processing. The sediment cores were taken at each site once during 2010. Sediment substrate samples were analyzed by removing biological material (debris); however, microorganisms were not accounted for with the 8000 micron (-3ɸ) sieve. Each sample and biological material were dried at 60 ºC for 48 hours. Once dried, each sample was then broken up and sieved through a series of dry sieves (-3ɸ, -1ɸ, 0ɸ, 1ɸ, 2ɸ, 3ɸ, and 4ɸ) for 15 minutes with the use of a Ro-Tap machine, and sieve samples were weighed to the nearest 0.01 mg (Loveland and Whalley 2000; Liebens 2001).

For sediment analysis a statistical package for analysis of unconsolidated sediments by sieving (GRADISTAT Version 4.0) was used to characterize sediment (percent mud, sand, and gravel) of each site along the Pamlico River. A soil pyramid was created to illustrate sediment distribution in the river. To analyze the similarities and dissimilarities among areas CLUSTER analysis (PRIMER v6 Statistical software) was used to identify differences in sediment, habitat, and abiotic factors. The groupings from the CLUSTER analysis were also used to compare mean CPUE and growth of both species.

LAB METHODS

Age, Growth, and Mortality (Loss Rate)

In the laboratory, each spotted seatrout (n=56) and red drum (n=87) were placed on a petri dish filled with water and the left and right saggital otoliths removed. Using a Konus crystal-45 dissecting scope, a small needle was inserted under the operculum behind the gills of the juvenile spotted seatrout and red drum to remove otoliths. The right otolith (oil immersion) was used to estimate juvenile spotted seatrout and red drum growth because of better outer ring resolution by the oil immersion (see otolith methods section).

Growth for the last seven days of spotted seatrout and red drum was analyzed to estimate short-term and recent growth within habitats. We chose this estimate under the assumption that collected spotted seatrout and red drum were at least in a specific habitat for seven days prior to capture. Similar studies have used this prior capture estimate (seven days) to examine short term or recent growth in fishes (Levin et al. 1997; Paperno et al. 2000). Daily annuli were viewed under an Olympus SZ-CTV light microscope with measurements taken from images captured by Image-Pro Discovery software package. Measurements of the last seven days were taken from the outer edge of the otolith to the inner 7th daily ring.

To estimate recent growth for juvenile spotted seatrout and red drum of the last seven days, the Modified-Fry method (L_i) was used to back calculate the instantaneous growth. Since individual fish species show allometric relationships in fish length to otolith radius growth and the present study had a low sample size, the L_i method was used (Wilson et al. 2009).

$$Li = a + exp\ (ln(L_{0p} - a) + (ln(L_{cpt} - a) - ln(L_{0p} - a)) \times \frac{(ln(R_i) - ln(R_{0p}))}{(ln(R_{cpt}) - ln(R_{0p}))})$$

Where a= fish length at otolith formation; exp= e raised to the nth power; L_{0p}=average fish length at first increment; L_{cpt}= fish length at capture; R_i= radius at age; R_{0p}= average otolith radius at 1st increment; R_{cpt}= otolith radius at capture.

The recent instantaneous growth for the last three and five days was also compared for each fish species within each habitat. Analysis of variance (ANOVA) was used to test whether there were significant differences in daily growth rates among habitats (α=0.05) and a Tukeys posthoc test was used to identify any differences.

Hatch date for both species was back-calculated by subtracting the estimated age (days) from the date of collection. Loss rate (*A*) was based on catch curve date (Ricker 1975) using the exponential declining model that includes emigration and declining catch efficiency.

$$N_t = N_0 e^{-Zt},$$

where N_t= number at age t; N_0= estimated number at hatching; Z= instantaneous mortality coefficient; and t= otolith estimated age. A regression was fitted to the at-age abundance date (ln(n+1)) with the slope of the line representing instantaneous mortality (Z). Estimates were then used to estimate a daily mortality rate. An analysis of covariance (ANCOVA) was used to test for differences in mortality rates among the habitats.

Each individual species was dried at 60 °C for 24 hours. Both the stomach and fish were weighed with an Accuseries-413 microbalance to the nearest 0.01 mg. The combined dry weight was determined and weight specific growth rates were calculated using the exponential regression model:

$$W_t = W_0 e^{gt},$$

where W_t = dry weight (g) at age t (d); W_0 = dry weight (g) at hatch; e = the base of the natural logarithm; g=weight –specific growth coefficient; and t = age.

An ANOVA was used to analyze condition (Fulton's Condition Factor (K)) of both species among area (West, Central, and East) and habitat (Sand, SAV, and detritus).

K was determined by:

$$K = (W/L^3) \cdot 100{,}000,$$

where W= the weight (g), L=length (mm), and 100,000 is a scaling constant.

Otolith Methods

Two otolith processing methods (polishing and immersion oil) were used to determine the readability and accuracy between the two methods. The left saggital otolith was embedded in an adhesive (Loctite 349). After embedding, the otoliths were sanded transversely using (400, 600, 800, and 1000 grit), polished with Buehler micropolish alumina (1.0, and 0.3 µm), then viewed under an Olympus BX41 microscope for ageing (twice). The right otolith was submerged in immersion oil for four weeks to allow for clearing. The submerged (right) otoliths were checked weekly for best annuli visibility and degradation (Secor 1991). After four weeks, the daily rings were counted twice by the same reader at different times. Furthermore, oil immersion otoliths (right) were used for recent growth (last seven day) calculations because of better outer ring resolution compared to polished otoliths (left). Because the first otolith ring is not formed until day two or three of life, three days were added to every otolith counted for spotted seatrout (Powell et al. 2000). However, counting red drum sagittal annuli is difficult, having underestimated the actual age by 21 days, and differences in form and structure of the left and right sagittae have not been well documented (Peters and McMichael 1987; David and Isely 1994; Stewart and Scharf 2008); no adjustments in counts were added to the age of red drum. Otoliths that were unable to be read were not used for ageing analysis. A paired t-test was used to compare the two age estimates within the polishing method and oil immersion method. If significant, a third count was conducted.

The precision or percent error between the two methods of aging was analyzed using the coefficient of variation:

$$CV = 100 \times SD/mean,$$

where CV= coefficient of variation; SD= standard deviation divide by the mean of the counts; and the percent error contributed by each aging estimate:

$$D = CV/\sqrt{R},$$

where D= percent error; CV = coefficient of variation; and R= the number of replicate interpretations square.

Diet Analysis

Diet was quantified using an index of relative importance (IRI) by combining prey weight, prey count, and prey frequency of occurrence (Pinkas 1971). Although IRI has received criticism (Hansson 1998), the index allows comparisons to other studies, provides a single measure of the diet, and is less biased than weight (W), volume (V), frequency (F) or number (N) alone (Cortes 1997; Cortes 1998). For the weight measure, the stomach contents of individual predators were determined by the proportional weight of each prey category following the fractionation method (Carr and Adams 1973). The stomach contents were poured through six mounted sieves 2000, 850, 425, 250, 150, and 75 µm, which were rinsed with water for approximately one minute. The contents were identified and enumerated by sieve size to lowest practical taxonomic level. After identification and enumeration, the contents of the stomach were aggregated per species, area (West, Central, East), and habitat type (Sand, SAV, detritus) and placed in a pre-weighed metal pan, and then dried at 60^{o}C for 24 hours. After drying, the pans containing the contents were weighed to within four significant digits (0.0001g) to determine gross weight.

Cumulative prey curves for each species were used to determine whether an adequate number of stomachs had been examined to describe the diet (Ferry and Caillet 1996). The sampling order in which stomachs were analyzed was randomized 100 times to reduce the bias resulting from sampling order. The mean number of new prey categories found in the stomachs was then plotted against the total number of stomachs analyzed, and the asymptote of the curve represented the minimum sample size required to adequately describe the diet (Ferry and Caillet 1996). We used the method outlined by Amundsen et al. (1996) to characterize the different feeding strategy of red drum and spotted seatrout (Fig. 2). Prey specific abundance (P_i) was plotted against frequency of occurs (%FO) where P_i was calculated as the number of $prey_i$ divided by the total number of prey in the stomachs that contained $prey_i$, expressed as a percentage. The prey located at the upper right of the diagram indicated a specialized diet (i.e., a narrow niche breadth) whereas prey located along or below the diagonal from the upper left to the lower right of the plot represented a broad niche breadth.

To evaluate diet similarity between red drum and spotted seatrout, the mean percentage composition by number for each prey group was calculated for each species, location (West Central, East), and habitat (SAV, sand, detritus). We used Primer-E software (Clarke and Gorley 2006) for multivariate analysis: fish groups were treated as samples and prey groups as variables. Square-root transformation was performed, and data were converted to a Bray–Curtis matrix for analysis. We conducted an analysis of similarity (ANOSIM), nonmetric multidimensional scaling (NMDS) and similarity percentages (SIMPER) methods (Clarke and Warwick 2001). ANOSIM is based on rank similarities between samples in the Bray–Curtis matrix and estimates a global test statistic (R). This R statistic represents the differences between sample groups compared to differences between replicates within sample groups (Clarke 1993). We considered pairwise R values 0.25 to represent substantial overlap / similarity, values 0.26–0.5 indicated moderate similarity, and values > 0.5 suggested little to no overlap between groups. The 2-D representation of NMDS results was considered usable when stress <0.2 (Clarke 1993). We used SIMPER to identify which prey species contribute the most to diet similarity within a fish group and diet differences between fish groups.

Affect of Community Composition on Two Species

PRIMER v6 Statistical software (Clarke and Gorley 2006) was used to determine if the presence of juvenile spotted seatrout and red drum had an effect on fish community structure.

Each sample was classified into one of three categories 1) those containing spotted seatrout, 2) red drum, and 3) spotted seatrout and red drum. The analysis was conducted on presence/absence data, which gives the less abundant species the same weight as more abundant ones. Analysis of similarities (ANOSIM) test was used to identify differences among the three categories. Non-metric multidimensional scaling (NMDS) test was used to visualize similarities among the three categories on the bases of Bray-Curtis similarity test.

RESULTS

Environmental Data and Sediment Analysis

Water quality parameters followed the expected seasonal patterns. Water temperature (°C) during months of August to November followed a downward decline. In contrast, dissolved oxygen (mg/L) and salinity (ppt) had lower levels occurring in August and then increased by the end of the sampling season (November). Water temperatures ranged from 11.4 to 32.8 °C and had a mean of (20.7±6.0) (Table 1). Wind was predominantly (<0 mph) calm 33% of the time, with speed significantly different throughout area and East area experience the highest monthly wind speeds. Conversely, pH stayed fairly constant values throughout months (Table 1).

Dissolved oxygen, pH and salinity differed among areas, but not habitat (Table 2). The highest levels of dissolved oxygen and pH existed in the Central area; the most variable levels occurred in the East (Fig. 3 and Fig. 4). Salinity followed the expected pattern and was consistent among areas (Fig. 5). Wind speed was most variable and differed between the Central and East areas (Fig. 6). Dissolved oxygen, pH, salinity, and wind were generally constant among habitats (Fig. 7-10).

Sediment Analysis and Habitat Distribution

Each area had specific sediment characteristics: the West area had the highest amount of gravel, Central had the highest biological matter, and East had the largest amount of silt (Fig. 11). Sand was the predominant sediment type throughout the sampling area and sediment size increased along a downstream gradient (Fig. 11 and 12). The results from the Cluster analysis showed that four groups existed throughout sites sampled along the river. Group one (G1) was comprised of site (45), which was a sand and biologics habitat located in the Central area. It had a low level of similarity (<58%) when compared to all other sites (>50%) that were mostly broken into fine and medium sand (Fig. 13). Group two (G2) sites (37,60,33,57,49,39,50) were fine sand; Group three (G3) sites (31,53,54) were medium sand; and Group four (G4) sites (41,64,52,55,62) were medium sand (Table 3).

Sand was the predominant habitat where 45% of all sites characterized as 45% sand, 21% SAV, and 34% as detritus. The West area sites consisted mostly of sand (30%), detritus (35%), and SAV (35%). Sand (20%), detritus (60%), and SAV (20%) existed in the Central area, and sand (16%), detritus (3%), and SAV (81%) in the East area (Fig. 14). The percent of spotted seatrout caught was highest in SAV habitats in the East and for red drum it was highest in detritus habitats in the Central (Fig. 15).

Abundance and Distribution

Juvenile spotted seatrout (N=56) were collected from October to November of 2009 and August to November 2010. However, juvenile red drum (N=86) were only collected in 2010. Spotted seatrout size ranged from 30 to 160 mm TL and occurred in 24% of the samples. Red drum size ranged from 15 to 65 mm TL and occurred in 15% of the samples (Fig. 16).

Spotted seatrout CPUE was significantly different among areas (Fig. 17). Spotted seatrout CPUE was higher in the West than the East; red drum CPUE was highest in the Central area (Fig. 17). Spotted seatrout CPUE was significantly higher in SAV, whereas red drum CPUE did not differ significantly among habitats or areas (Fig. 17 and 18). In general, 57% of spotted seatrout were caught in SAV, 19% in detritus, and 24% in sand. For red drum, 52% were caught in detritus, 28% in SAV, and 20% in sand (Fig. 19). No spotted seatrout were collected in SAV areas in the East (Fig. 20). Red drum CPUE differed among areas with high catches in Central (detritus) area and no red drum were collected in detritus habitats in the West area (Fig. 20). Grouping CPUE of both spotted seatrout and red drum using the CLUSTER analysis showed no difference in mean CPUE of spotted seatrout among groups. However, Group 1, which conceded of only one site (45) in the CLUSTER did differ among groups for red drum (Table 4).

Size (TL) was significantly different for juvenile spotted seatrout among areas ($F_{2,55}$=4.06,p=0.0229), but was not for red drum caught during sampling ($F_{2,85}$=2.29,p=0.1077). The mean sizes µ ± (SE) of spotted seatrout was 66.5±4.4 mm and was 32.6±1.1 TL mm for red drum. The largest spotted seatrout and red drum were collected in the West area (Fig. 21). No spotted seatrout were collected in the East (SAV) and no red drum were collected in the West (detritus) or in the East (sand) habitats (Fig. 21).

Dry-weight showed a positive relationship to standard length for juvenile spotted seatrout and red drum (Fig. 22). Spotted seatrout dry-weights did not significantly differ among habitats (F_2=2.82,p=0.0703), but did for red drum (F_2=3.85,p=0.0256) in SAV compared to detritus. The condition factor (K), did not differ among habitats for spotted seatrout (F_2=1.42,p=0.2527) or by area (F_2=0.7205,p=0.7205) (Fig. 23 and 24). The same pattern held true for red drum where condition (K) did not differ among habitats (F_2=0.85,p=0.4305) or for areas (F_2=1.18,p=0.3137 (Fig. 23 and 24).

Hatch Date and Age Distribution

The age of spotted seatrout ranged from 50 to 160 d (µ=89.2 ± 4.0). Red drum age ranged from 40 to 120 d (µ=60.1 ± 1.8) (Fig. 25). The age estimates were positively related to dry-weight (Fig. 26). Juvenile spotted seatrout and red drum hatch date distribution showed that spotted seatrout spawning occurred from April to September and red drum spawning was from July to October. June was the peak spawning time period for spotted seatrout where as, peak spawning occurred in August for red drum (Fig. 27). The hatch day estimates of this study co-insides with acoustical studies, which hear drumming spotted seatrout and red drum during this time of year in North Carolina (Sprague et al. 2000; Luczkovich et al. 2008b).

Otolith Method Comparison

The otolith preparation methods produced different results for spotted seatrout, but was not significantly different for red drum (Table 5). For spotted seatrout, the oil immersion method estimated higher ages, whereas the polishing method estimated lower ages of fish (Fig. 28). For red drum (65-d, <30 mm TL) the oil immersion estimated lower age for fish and (65-d, >30 mm TL) the oil immersion estimated higher age than polishing estimates (Fig. 29).

Growth and Mortality (Loss Rate)

The instantaneous growth rates during the last seven days of life were not significantly different within each habitat for either species (Table 6). Spotted seatrout showed no difference in last three day growth ranging from 0.0143 to 0.129, five day ranging from 0.013 to 0.101, and seven day ranging from 0.011 to 0.084 in relation to habitat. Furthermore, red drum growth did not differ during the last seven day growth within each habitat collected. Red drum showed no significant differences within habitat in last three day growth ranging from 0.004 to 0.201, five day ranging from 0.003 to 0.148, and seven day ranging from 0.003 to 0.171. Furthermore, there was no difference in three, five, and seven day growth among the four groups defined by the CLUSTER analysis (Table 7).

Daily spotted seatrout mortality rates from the field season (2009-2010) were 1.5%/d in detritus, 1.7%/d in sand and 1.4%/d in SAV habitats. There was no significant difference (F_2 =0.04, p=0.9646) in mortality rates among habitats. Daily mortality rates for red drum within habitats in 2010 field season were in detritus (4.4%/d), in sand (2.3%/d), and SAV (4.9%/d); there was no significant difference among habitats (F_5=1.19,p=0.4131).

Affect of Community Composition on Two Species

The one-way ANOSIM and non-metric multidimensional scaling (MDS) indicated that the fish community did not differ in the presence of spotted seatrout or red drum or both species present (Global R = -0.058, p=94.2%) (Fig. 30 and 31). The major of fish species contributing to fish community among areas were bay anchovy (*Anchoa mitchilli*), inland silverside (*Menidia beryllina*), and spot (Table 8).

Diet Composition

The cumulative prey curve showed a well defined asymptote for both species (Fig. 32), indicating that the total sample size was adequate to describe the diets of red drum and spotted seatrout using the 13 prey categories (Table 9). The percentages of empty stomachs varied between the species. Stomachs that contained no food (n=81) for red drum and (n=45) for spotted seatrout. The analysis identified 10 groups of prey consisting mostly of crustaceans for both species. For red drum, amphipods, isopods, and mysids were the primary prey in the diet and collectively occurred in 62.9% of the stomachs containing food (Table 10). These prey items also accounted for >70.0% of the diet weight. Bivalves, insect nymphs, and gastropods were absent from the red drum diet but present in the spotted sea trout diet. For spotted seatrout mysids were the most dominant prey item occurring in 24.6% of the stomachs containing food and accounting for >50% of the diet biomass (Fig. 33). Anchoa *mitchilli,* fish eggs and mysids (IRI of 77%) occurred in 55% of spotted seatrout stomachs containing food. Polycheates were present in 27.4% of the red drum stomachs containing food, but were absent in spotted seatrout diet.

The results of the two way ANOSIM on diet composition showed that there were no differences in diet among the sampling areas ($R = 0.021$, $P < 0.27$). However, there were differences in diet between species ($R = 0.274$, $P < 0.001$). Most of the differences in diet (SIMPER) were driven by mostly by isopods, mysids and amphipods (Fig. 34). Collectively these three prey were responsible for 47.6% of the dissimilarity.

DISCUSSION

Habitat

Juvenile spotted seatrout abundance during sampling did differ by area (West, Central, and East) and habitats along the river. The habitat effects on spotted seatrout occurred in SAV where abundance was highest, but no differences existed for red drum in area or habitat. Studies in other systems have shown that distribution patterns and abundances of spotted seatrout are influenced by SAV densities in an aquatic system. For example, a study in western Florida showed juvenile spotted seatrout and gray snapper (*Lutjanus griseus*) had a preference for basin areas and SAV densities exceeding 1,000 shoots per square meters (Chester and Thayer 1990). Additionally, spotted seatrout, red drum and other fishes are more associated with complex vegetated areas than non-vegetated bottoms or marsh edges

(Stunz and Minello 2001; Nagelkerken and Van Der Velde 2002; Neahr et al. 2010). A predator prey study on juvenile red drum demonstrated that complex habitats help lower mortality rates of wild and reared red drum when pinfish (*Lagodon rhomboids*) and spotted seatrout predators were present (Stunz and Minello 2001).

A resource map of SAV complex habitats along Pamlico River designated cover classifications of SAV as dense (70-100%), patchy (5-70%) and absent (0-5%). The Pamlico River is considered to have dense (66.8 ha) and patchy (21.0 ha) areas of SAV (NCDENR 2011). Furthermore, proportionally the Pamlico River has 14.8% SAV and the majority of the river is considered to be absent coverage (Joey Powers un-published data). This may explain why juvenile red drum had higher abundance in detritus compared to SAV and sand. The difference between spotted seatrout and red drum abundances among habitats may reflect that red drum are more sensitive to density and size of complex habitats than spotted seatrout. The results from this study suggest that habitat complexity has greater influence on juvenile red drum recruitment than to spotted seatrout.

The purpose of this study was to analysis not only SAV habitats, but other substantial habitats (detritus and sand) where spotted seatrout and red drum occur. Even though young-of-year red drum prefer seagrass and are found in low abundance in areas without seagrass, they may utilize other areas when seagrass is limited (Stunz et al. 2002a). Other studies suggest that when SAV or seagrass meadows are less available, red drum will recruit to the next most complex habitat (Series 1992; Baltz et al. 1993; Stunz et al. 2002a). A similar results was found in this study, where the next most complex habitat studied was detritus. Juvenile red drum had higher abundance in detritus compared to SAV.

Spotted seatrout individuals (27%) collected in the seine were >80 mm TL and 5% of collected red drum individuals where >50 mm TL, which may imply individuals of a certain size may have not been susceptible to the gear or movement between habitats was occurring. This relates to other studies where >30 mm fish are rarely collected in the gear used (seine or benthic sled) (McMichael and Peters 1989; Stunz et al. 2002a). Comparing detritus and/or organic matter habitats to seagrass beds for recruitment of juvenile spotted seatrout and red drum are missing in other studies. My study attempted to enlighten and investigate this issue by comparing two habitats that are considered habitats (SAV and sand) to one not usually considered a habitat (detritus).

The contingent theory may explain why the CPUE patterns of juvenile spotted seatrout and red drum existed along the river. The Pamlico River empties into Pamlico Sound and most salinities that were measured are not favorable for spawning ≤15.0 ppt (Saucier and Baltz 1993; Rooker and Holt 1997). Spotted seatrout and red drum spawning could actually occur in Pamlico Sound, with recruits migrating into the river (sink) while others stay out in the Sound (source). Kraus and Secor (2004) showed that after spawning, white perch (*Morone americana*) offspring recruited to freshwater, while others stayed in brackish habitats. They also concluded that having these contingent individuals may help with maintaining population levels for reproduction, which could be occurring with spotted seatrout and red drum in Pamlico River.

Environmental Data

Within the sampled areas (West, Central, and East) and habitats (detritus, sand, and SAV) the environmental parameters did not differ except for pH and dissolved oxygen, which had high values in Central and the most variability in the West. Salinity had high values in the East and most variability in the West. Spotted seatrout occur in 18-32 ppt but are most abundant at 20 ppt

(McMichael and Peters 1989; Kupschus 2003; Wuenschel et al. 2004). In comparison, red drum select areas of salinity of 0-34 ppt and moderately higher 35 ppt (Crocker et al. 1981; Peters and McMichael 1987; Adams and Tremain 2000). In this study, salinities ranged from 0.4 to 21.4 ppt, with most spotted seatrout and red drum on average collected in salinities of 10.1 ppt and 12.5 ppt, respectively.

Another important environmental parameter essential for fish and aquatic life is dissolved oxygen. Dissolved oxygen ranging from 4.2 to 8.8 mg/L is fundamentally important in supporting juvenile spotted seatrout, with 6.5 mg/L being the optimal average for growth (Baltz et al. 1998). Juvenile red drum are found in dissolved oxygen ranging from 3.7 to 10.2 mg/L with 7.9 mg/L being the average ideal for growth (Baltz et al. 1998). In my study, dissolved oxygen ranged from 2.15 mg/L to 12.12 mg/L, with higher abundance of juvenile spotted seatrout caught at 7.9 mg/L. On the other hand, juvenile red drum were caught in higher abundances at 9.1 mg/L. The dissolved oxygen ranges of my study covered the essential ranges that spotted seatrout and red drum need for growth.

Age, Growth, and Mortality (Loss Rate)

Instantaneous growth rates (last 3, 5, and 7-d) were not significant among habitats for both species, which may imply that they are not staying in a habitat long enough to make a difference in growth. This may also indicate that the habitat effect is not strong enough to affect habitat specific response. However, the highest overall growth rates were found in SAV for spotted seatrout and detritus for red drum. The growth rates for both species observed in this study are similar and followed the same patterns to others (Peters and McMichael 1987; McMichael and Peters 1989; Stunz et al. 2002b).

Acquiring the most cost effective and precise techniques for calculating growth and age estimates for fish species is essential in fisheries science. The technique of polishing otoliths has been used in numerous studies for aging estimates (McMichael and Peters 1989; Prentice and Dean 1991; Murphy and McMichael 2003) as well as the technique of embedding otoliths in oil immersion for a period of time to clear (May and Jenkins 1992; Morales-Nin and Aldebert 1997; Grorud-Colvert and Sponaugle 2006). However, validation between polishing and oil immersion has not been determined in sciaenid species. My study suggests that the two methods (polish and oil) show similar results between collected juvenile spotted seatrout and red drum.

Most spotted seatrout and red drum studies use the polishing method (David and Isely 1994; Baltz et al. 1998; Powell et al. 2000). My study showed that age estimates between both methods for spotted seatrout were significant, with oil immersion having the best percent error and estimating a higher age than polishing. This suggests that oil immersion should give a better estimate of age for juvenile spotted seatrout 20-140 mm SL. The age estimates between both methods for red drum were not significant and the percent error was almost equal between the two. Because this is the case, both methods maybe adequate when aging 10-50 mm SL fish. The use of oil immersion is easier because the otolith is submerged in oil immersion for four weeks to clear, and then aging can proceed. The otolith submerged longer than four weeks have less visible rings because the otolith starts to degrade (Secor 1991). Last and most important it produces similar results to traditional embedding techniques (polishing) for red drum, which can be difficult to age.

During the sampling period, daily loss rate was highest in sand habitats (1.7%/d) compared to the more complex SAV and detritus. These results reinforce laboratory and field study experiments, which show juvenile spotted seatrout and red drum are found in higher abundances and preferring complex habitats than ones with less structural complexity (Stunz et al. 2001; Neahr et al. 2010). In this study, movement to these more complex habitats may have possibly helped reduce mortality of spotted seatrout. Our estimates of loss rate were slightly higher than estimates from a study with similar size fish (16-144 mm) (Rutherford et al. 1989). A different situation exists with red drum; higher daily mortality occurred in SAV habitats (4.9%/d) compared to the other two habitats. One aspect may be that more SAV habitat existed toward the mouth of the river. If red drum recruit into the river from the sound, the SAV may be the first habitat where recruiting individuals occupy. In Tampa Bay Florida, Peters and McMichael (1987) found that red drum size increased as collection progress upstream of the river. Similar results were reported by Stunz and Minello (2001) studying captive and wild red drum, where high mortality occurred in un-vegetated areas and medium levels in seagrass. Furthermore, it may be a size-selective mortality because smaller red drum have a wider range of predators than their larger counterparts spotted seatrout within these habitats (Sogard 1997).

Affect of Community Composition on Two Species

In my study, the presence of spotted seatrout and red drum had no apparent effect on community structure within the sampling area. Other studies have suggested that time of year has an effect on community structure and the dominant species of that community. Species co-occurrence can make species communities unique (Akin et al. 2003; Murphy and Secor 2006). An example of this is a study by Series (1992), who found spotted seatrout and silver perch co-occurring in the same marsh areas. However, this was not observed in the present study and could be due to other influences. Rakocinski at el. (1992) found shifts in community structure and dominating fish species during the spring, followed by changes in water temperature and decrease in dissolved oxygen. In the present study, neither spotted seatrout nor red drum were ever the dominant species collected and because of their low numbers they may not affect the community.

Juvenile spotted seatrout and red drum distributions could be contributed to food association or fish recruitment with wind direction. Hamner et al. (1988) found that plankitvorus fish species were highly associated with the windward side of coral reefs waiting for zooplankton (food) to float by. Elson (1939) analyzed how speckled trout (*Salvelinus fontinalis*) moved in different water currents and found that fish placed in even current showed some orientation and distribution as they swam against the current. Fish may recruit to an area along the river because of wind driven inshore currents, which may have an effect on distribution of young of year red drum (Stewart and Scharf 2008). In Elson's study (1939) it was established that in a low flow system, fish typically randomly wandered. My study could be during a period of low water flow, with spotted seatrout and red drum randomly wandering to habitats. In my study, juvenile spotted seatrout were larger (30-160 mm) and had higher abundance in SAV habitats compared to juvenile red drum that were smaller (15-65 mm) and more abundant in detritus habitats. My results suggest that more research is needed to fill the gap in how juvenile spotted seatrout and red drum relate to detritus habitats compared to other habitats and how they may benefit from it.

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Table 1: Average monthly water quality estimates (±SD) for habitats studying along the Pamlico River, North Carolina from August through November (2009-2010).

Month	Area	Wind (m/s)	Water temp (°C)	D.O. (mg/L)	pH	Salinity (ppt)
August	Detritus	1.5(1.4)	30.3(0.6)	7.5(3.2)	8.4(0.5)	8.8(3.0)
	Sand	1.8(1.6)	30.3(0.9)	7.8(1.0)	8.4(0.2)	8.1(3.2)
	SAV	2.4(2.7)	30.5(1.1)	7.9(1.1)	8.4(0.3)	7.1(2.0)
September	Detritus	1.6(2.5)	26.2(2.0)	7.1(1.4)	8.1(0.2)	11.2(2.9)
	Sand	2.4(2.2)	26.5(1.1)	7.7(1.4)	8.5(0.2)	10.3(3.6)
	SAV	1.3(1.6)	26.6(1.3)	7.9(1.3)	8.3(0.2)	9.1(2.6)
October	Detritus	2.7(1.8)	19.5(3.0)	8.5(1.9)	7.7(0.4)	10.7(4.3)
	Sand	2.3(2.7)	19.2(2.6)	8.3(1.5)	8.1(0.4)	11.5(4.8)
	SAV	1.5(1.4)	18.6(2.4)	8.2(1.6)	8.0(0.4)	10.8(5.6)
November	Detritus	1.5(1.7)	15.4(1.5)	8.8(1.2)	8.2(0.2)	13.4(3.4)
	Sand	1.5(1.4)	15.4(1.5)	8.9(1.3)	8.3(0.1)	12.1(4.7)
	SAV	0.9(0.9)	15.3(1.6)	8.9(1.5)	8.2(0.2)	12.7(4.3)

Table 2: Analysis of variance comparing water temperature, dissolved oxygen, pH, and salinity by area and habitat along the Pamlico River, North Carolina (2009-2010).

	Area			Habitat		
Environmental parameters	d.f.	F	*p*	d.f.	F	*p*
Water temperature (°C)	2	1.21	0.29	2	0.03	0.97
Dissolved oxygen (mg/L)	2	10.57	0.0001	2	0.47	0.62
pH	2	11.90	0.01	2	0.19	0.82
Salinity (ppt)	2	58.06	0.0001	2	2.79	0.06

Table 3: The four groups (G) of CLUSTER analysis representing mean percent sediment type for each grouping in Pamlico River, North Carolina.

Sediment	G1	G2	G3	G4
Biological material	22.0	.19	0.30	.52
Silt	15.6	9.4	2.9	1.5
Very fine sand	28.3	21.3	3.6	4.2
Fine sand	18.7	32.8	5.0	79.5
Medium-fine sand	9.2	24.4	26.6	50.7
Medium sand	5.5	7.7	44.3	20.4
Course sand	1.2	2.8	16.8	6.1
Gravel	0.02	8.7	2.4	1.5

Table 4: The four groups (G) of CLUSTER analysis representing mean CPUE of spotted seatrout and red drum for each grouping in Pamlico River, North Carolina.

				Mean CPUE			
Species	*N*	F	d.f.	G1	G2	G3	G4
Spotted seatrout	192	1.41	3	(0.0024)^a	(0.0020)^a	(0.0015)^a	(0.0028)^a
Red drum	192	10.36	3	(0.0127)^a	(0.0023)^b	(0.0012)^b	(0.0016)^b

Table 5: Analysis comparing the ages of oil immersion and polishing methods of juvenile spotted seatrout (30 to 160 mm TL) and red drum (15 to 65 mm TL). Where CV= coefficient of variation; D= percent error; r^2= the number of replicate interpretation square; t= t-value; d.f. =degrees; *p*=probability.

Method	Regression	Percent error $D=CV/\sqrt{R}$	r^2	t	d.f.	*p*
Spotted seatrout						
Oil	y=0.9858x+40.353	5.53	0.79	-2.43	44	0.0195
Polishing	y=1.0101x+30.36	7.85	0.75			
Red drum						
Oil	y=1.7734x+14.729	6.57	0.76	0.29	82	0.7748
Polishing	y=1.2015x+31.539	6.33	0.51			

Table 3: Back-calculation of the last 3, 5, and 7 days of growth and average instantaneous growth rate per day (±SD) of juvenile spotted seatrout and red drum in relation to habitat type.

Growth	d.f.	F	*p*	Instantaneous growth rate per day
Spotted seatrout				
3 day growth	2	0.04	0.9601	0.036(0.024)
5 day growth	2	0.06	0.9415	0.033(0.021)
7 day growth	2	0.06	0.9407	0.032(0.019)
Red drum				
3 day growth	2	2.71	0.0735	0.023(0.033)
5 day growth	2	2.83	0.0655	0.020(0.026)
7 day growth	2	2.80	0.0676	0.018(0.024)

Table 4: Back-calculation of the last 3, 5, and 7 days of growth of juvenile spotted seatrout and red drum using the groupings from the CLUSTER analysis.

Growth	d.f.	F	*p*
Spotted seatrout			
3 day growth	3	0.65	(0.58)[a]
5 day growth	3	0.65	(0.58)[a]
7 day growth	3	0.73	(0.54)[a]
Red drum			
3 day growth	3	2.10	(0.10)[a]
5 day growth	3	1.93	(0.13)[a]
7 day growth	3	1.75	(0.16)[a]

Table 5: The average per seine haul CPUE of the three most important species in each section in Pamlico River, NC. Fish were collected twice a month using a beach bag seine from August to November 2009-2010 (Mabe 2012).

Species	Section	Per seine haul abundance
M. beryllina	West	11.75
A. mitchilli		12.17
L. xanthurus		2.41
A. mitchilli	Central	18.4
M. beryllina		8.52
L. xanthurus		3.34
A. mitchilli	East	17.82
M. beryllina		4.76
L. xanthurus		1.69

Table 6: Names, categories, and aggregated prey categories used in the diet analysis.

Category	Description
Amphipoda	Shrimp like in form
Fish	All fishes, remains and eggs (exclusively *Anchoa mitchilli*)
Calanoida	Calanoid copepods
Caridea	Shrimp
Cladocera	Small crustaceans
Gastropods	Snails
Insects	All larval and adult insects (almost exclusively dipteran larvae)
Isopoda	Small crustaceans
Mysidae	All mysid shrimps
Annelids	Polychaeta

Table 7: Diet composition of red drum and spotted sea trout in Pamlico River estuary. The diet indices are defined in the text (%FO, percentage frequency of; %N, percentage numerical abundance; %W, percentage Biomass; and % IRI, index of relative importance on a percent basis (%IRI). The unknown/other category includes unidentified items, sand, and plant material.

	Red drum				Spotted seatrout			
Category	%FO	%(W)	%N	%IRI	%FO	%(W)	%N	%IRI
Amphipoda	14.5	22.1	12.4	14.2	8.7	4.7	4.2	2.2
A. mitchilli	6.5	6.5	2.4	1.6	15.9	12.0	6.7	8.4
Bivalvia	0.0	0.0	0.0	0.0	1.4	1.4	0.4	0.1
Calanoida	4.8	1.8	2.9	0.7	5.8	2.3	4.6	1.1
Caridea	1.6	3.4	0.6	0.2	5.8	9.1	2.1	1.8
Cladocera	3.2	0.6	12.9	1.2	5.8	1.1	2.1	0.5
Diptera	1.6	3.4	0.6	0.2	4.3	1.6	3.5	0.6
Egg	4.8	2.5	2.4	0.7	14.5	3.6	12.3	6.4
Gastropoda	0.0	0.0	0.0	0.0	1.4	0.3	0.7	0.0
Insect nymph	0.0	0.0	0.0	0.0	2.9	7.2	0.7	0.6
Isopoda	21.0	27.7	24.7	31.3	5.8	4.1	1.4	0.9
Mysidae	27.4	21.5	35.3	44.4	24.6	51.8	60.0	77.1
Polychaeta	12.9	9.6	5.3	5.5	0.0	0.0	0.0	0.0
Unknown/Other	1.6	0.8	0.6	0.1	2.9	0.8	1.4	0.2

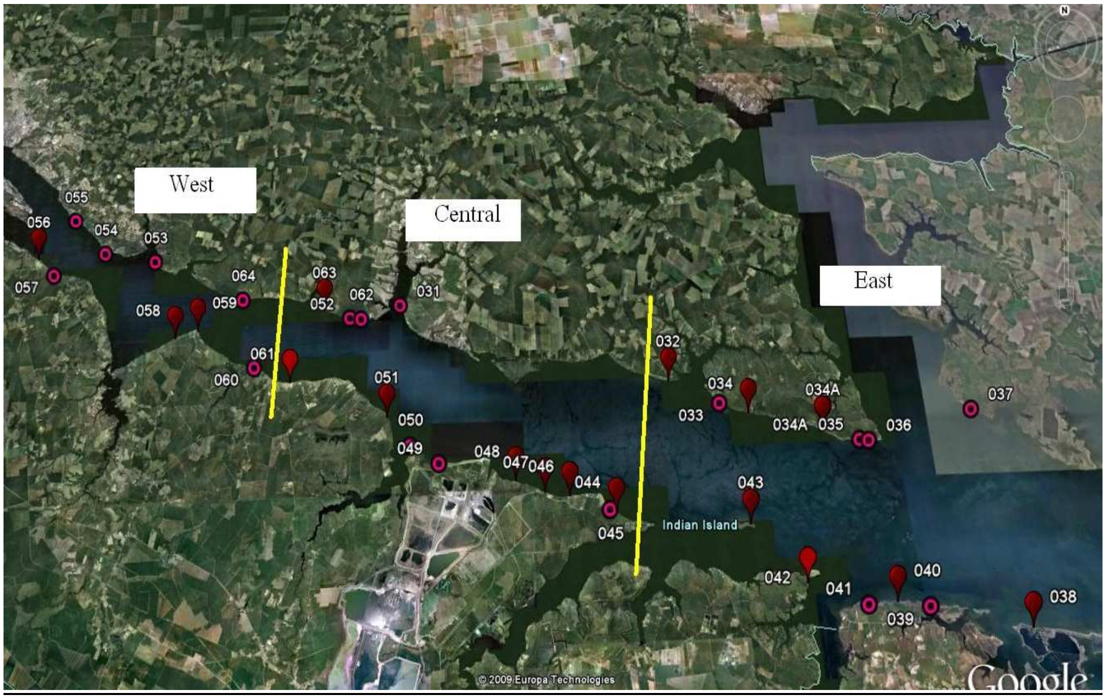


Figure 1: The 65 km study site of the Pamlico River from the Fork Point Island to the mouth of the Pungo River, North Carolina and the three sections West, Central and East and all 36 sub-sites. Red marks represented potential sites not selected by the random number generator, and pink circles (six stations within each section) represent sites that were included in the study.

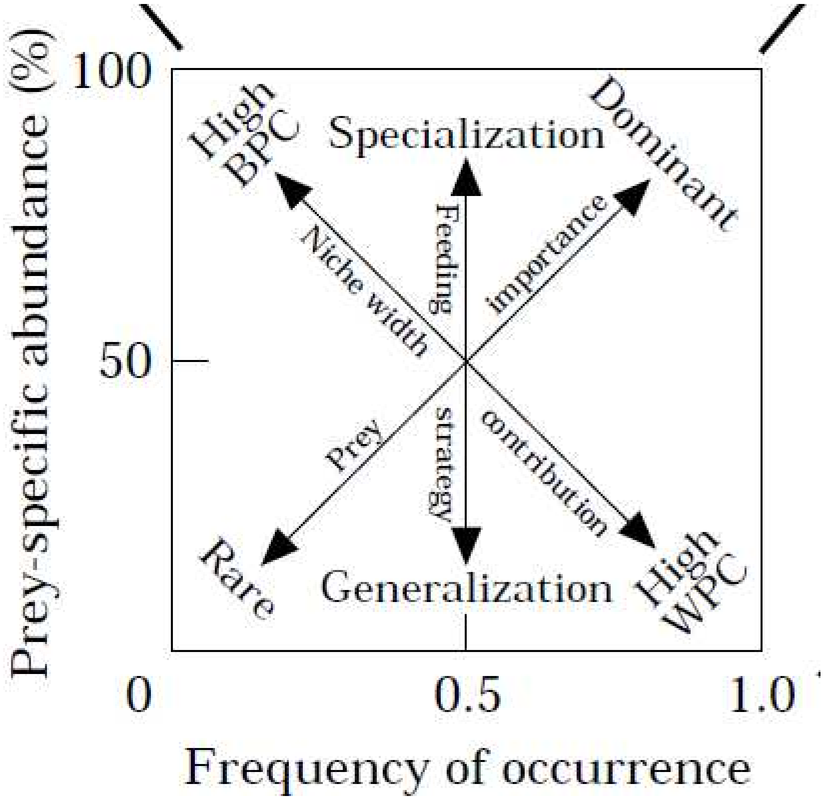


Figure 2. Two-dimensional diagram representing prey frequency of occurrence and abundance of a fish taxon (Amundsen et al. 1996).

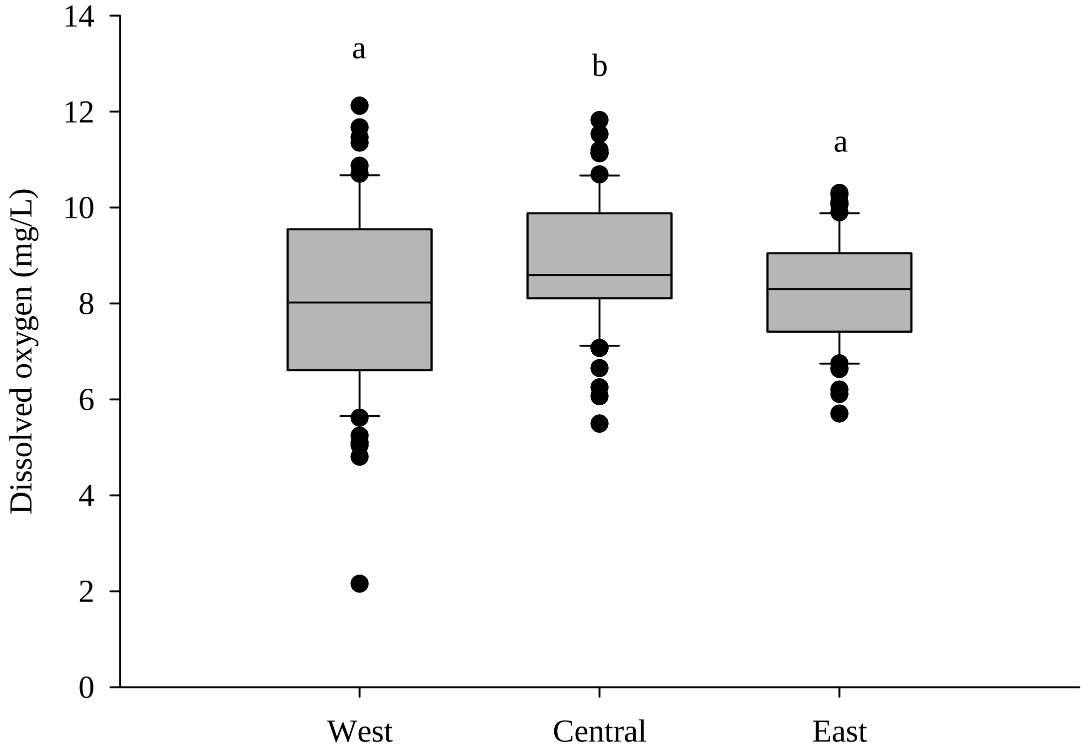


Figure 3. Mean dissolved oxygen (mg/L) from Fork Point Island, NC to the mouth of the Pungo River, NC sampled twice a month from August to November (2009-2010) using an 18-m long bag seine in Pamlico River, North Carolina. The dots represent outliers and the whiskers represent the minimum and maximum of 100% of the values.

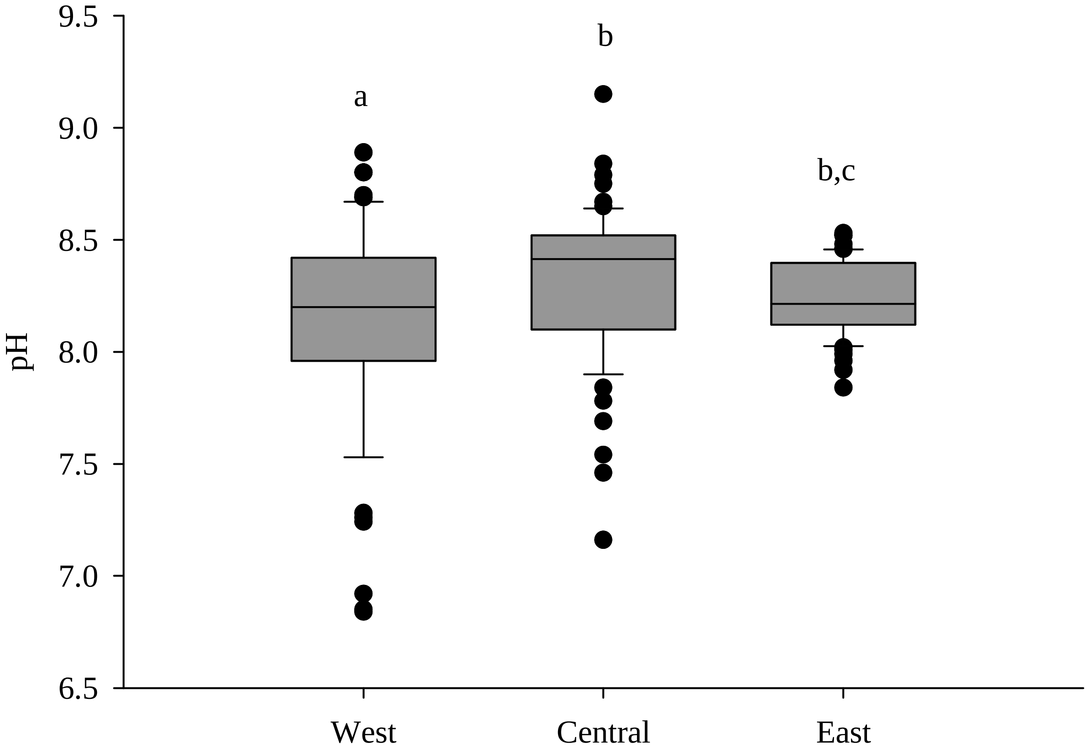


Figure 4. Mean water quality parameter pH from Fork Point Island, NC to the mouth of the Pungo River, NC sampled twice a month from August to November (2009-2010) using an 18-m long bag seine in Pamlico River, North Carolina. The dots represent outliers and the whiskers represent the minimum and maximum of 100% of the values.

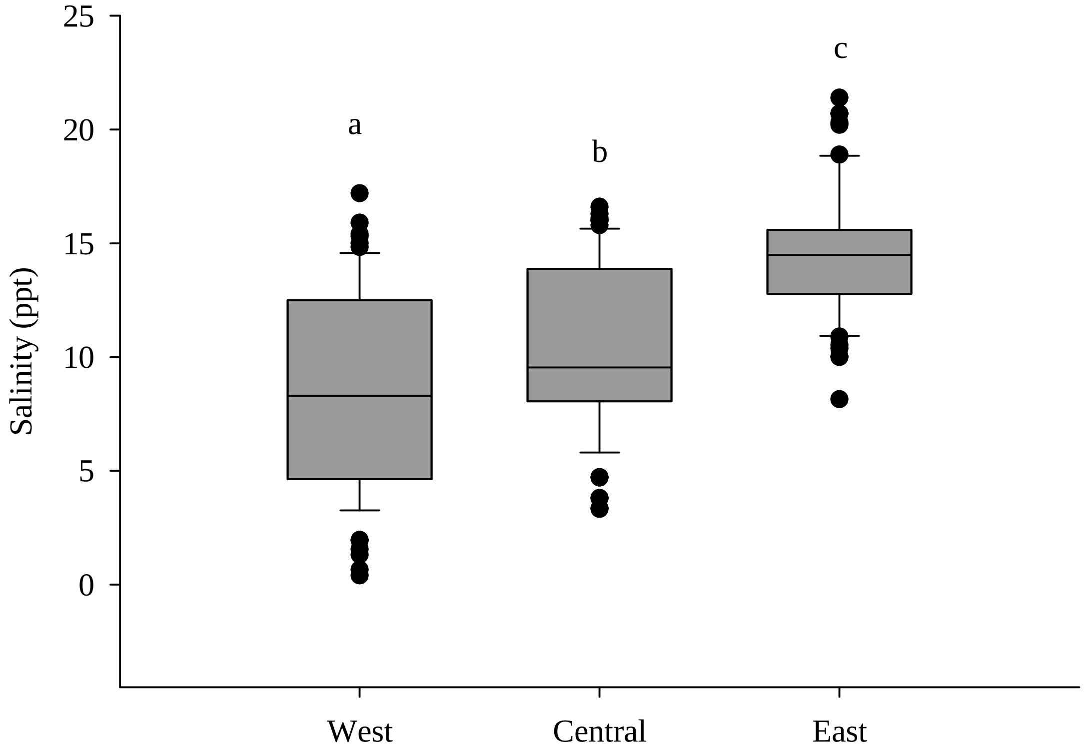


Figure 5. Mean salinity (ppt) water quality parameter from Fork Point Island, NC to the mouth of the Pungo River, NC sampled twice a month from August to November (2009-2010) using an 18-m long bag seine in Pamlico River, North Carolina. The dots represent outliers and the whiskers represent the minimum and maximum of 100% of the values.

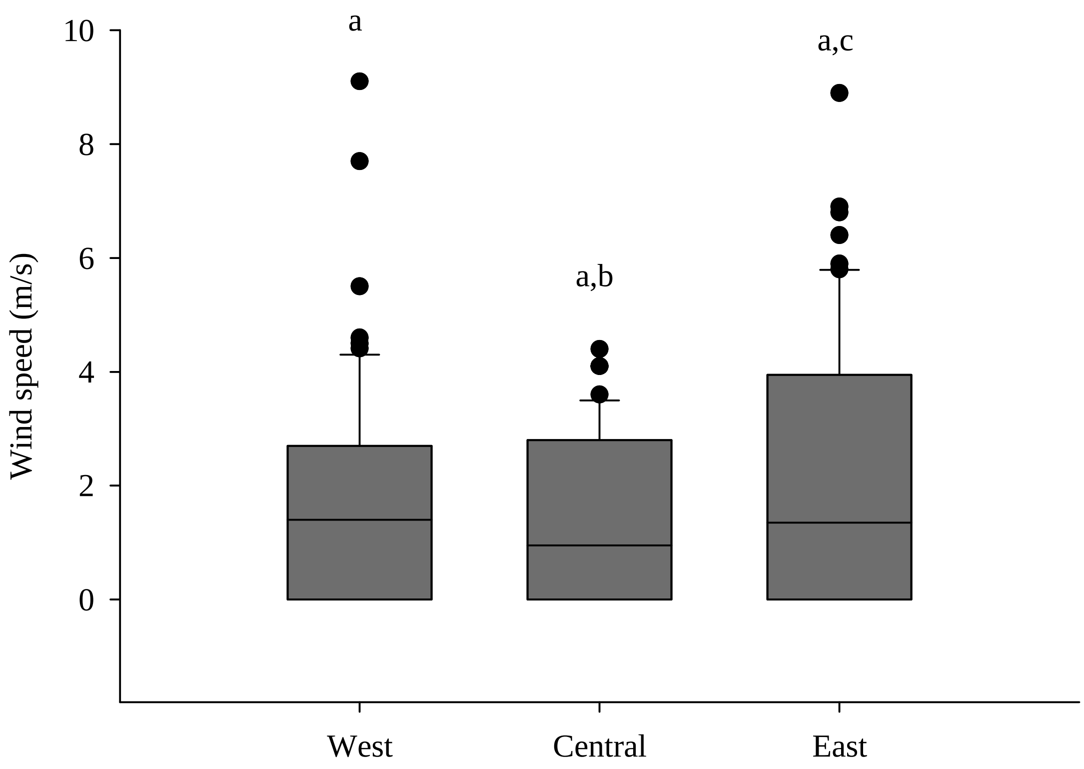


Figure 6. Mean wind speed (m/s) water quality parameter from Fork Point Island, NC to the mouth of the Pungo River, NC sampled twice a month from August to November (2009-2010) using an 18-m long bag seine in Pamlico River, North Carolina. The dots represent outliers and the whiskers represent the minimum and maximum of 100% of the values.

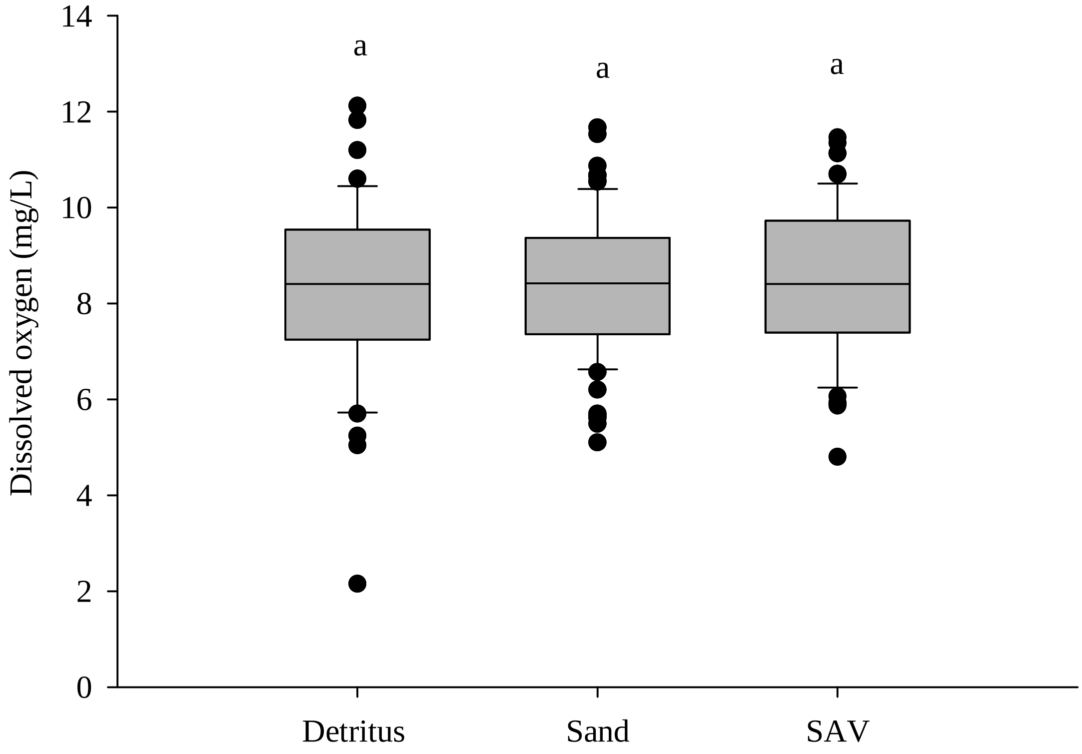


Figure 7. Mean dissolved oxygen (mg/L) of habitat from Fork Point Island, NC to the mouth of the Pungo River, NC sampled twice a month from August to November (2009-2010) using as 18-m long bag seine in Pamlico River, North Carolina. The dots represent outliers and the whiskers represent the minimum and maximum of 100% of the values.

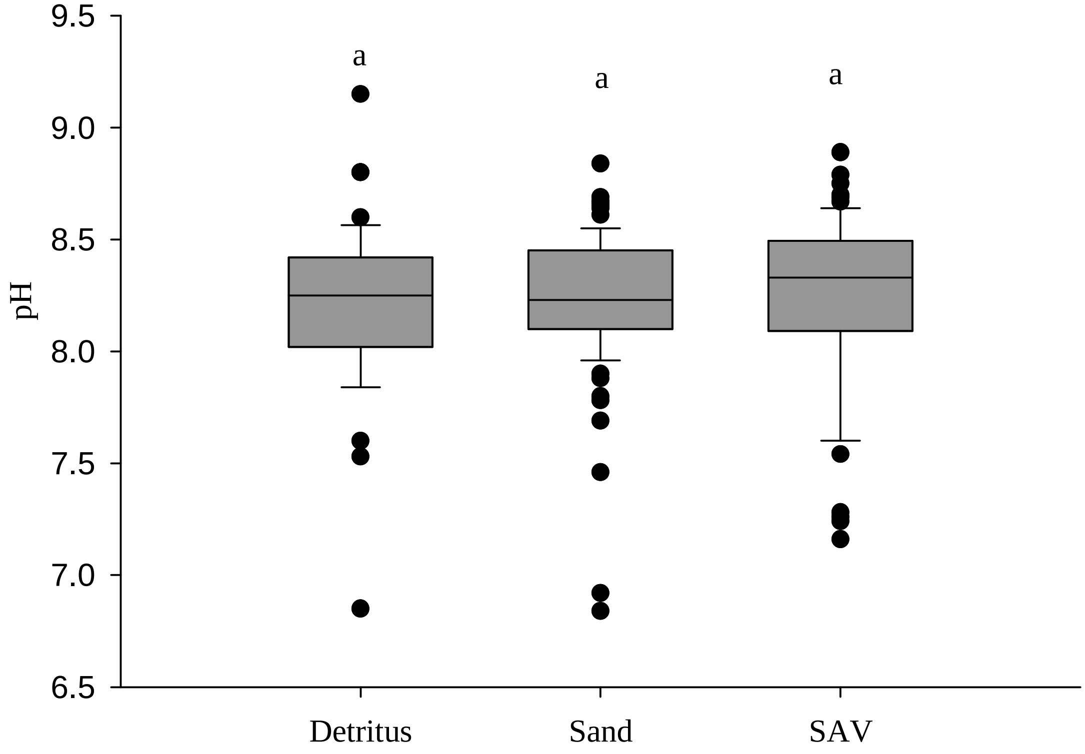


Figure 8. Water quality parameter pH of habitat from Fork Point Island, NC to the mouth of the Pungo River, NC sampled twice a month from August to November (2009-2010) using an 18-m long bag seine in Pamlico River, North Carolina. The dots represent outliers and the whiskers represent the minimum and maximum of 100% of the values.

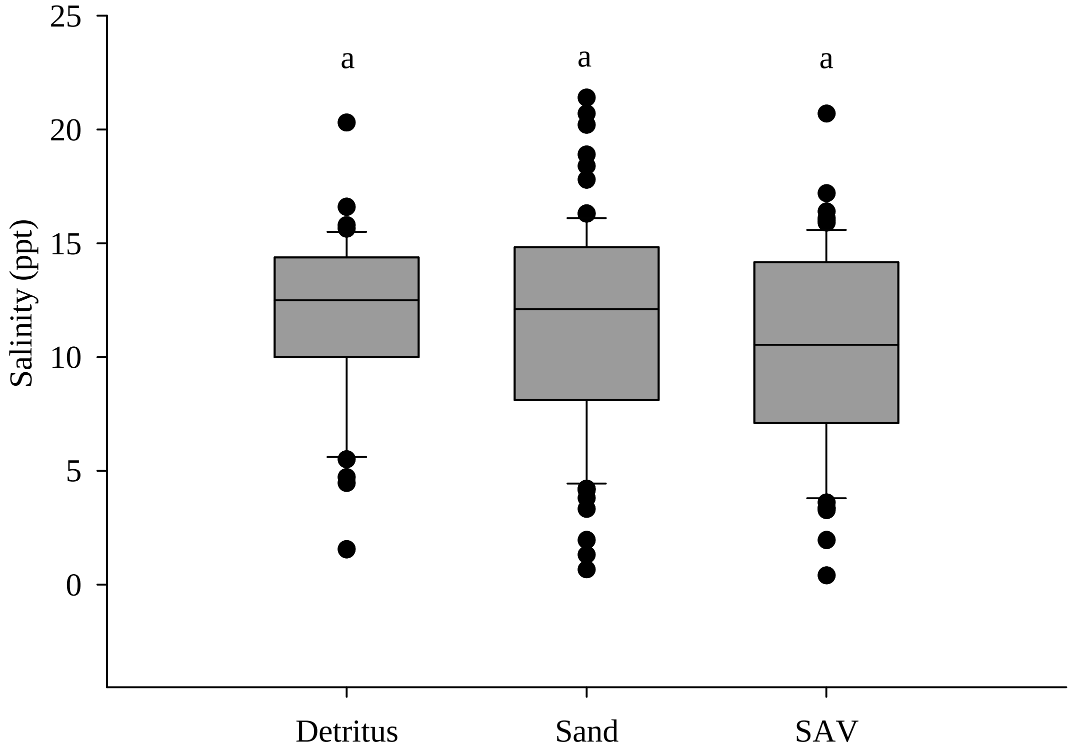


Figure 9. Mean salinity (ppt) water quality parameter of habitat from Fork Point Island, NC to the mouth of the Pungo River, NC sampled twice a month from August to November (2009-2010) using an 18-m long bag seine in Pamlico River, North Carolina. The dots represent outliers and the whiskers represent the minimum and maximum of 100% of the values.

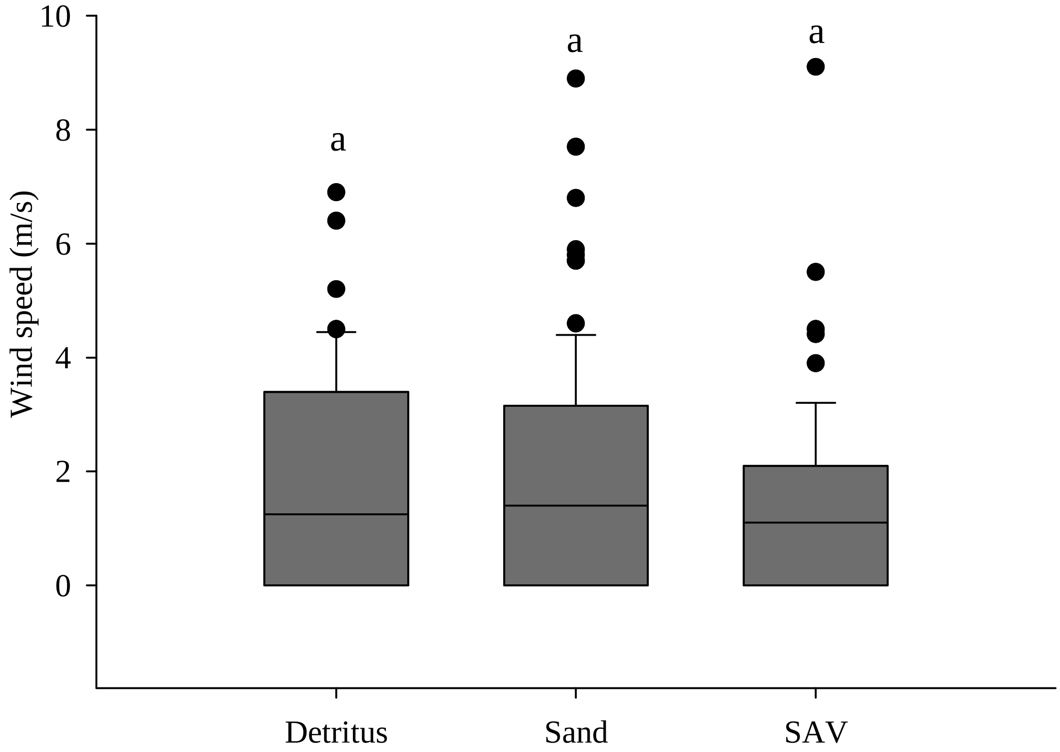


Figure 10. Mean wind speed (m/s) water quality parameter of habitat from Fork Point Island, NC to the mouth of the Pungo River, NC sampled twice a month from August to November (2009-2010) using an 18-m long bag seine in Pamlico River, North Carolina. The dots represent the minimum and maximum of 100% of the values.

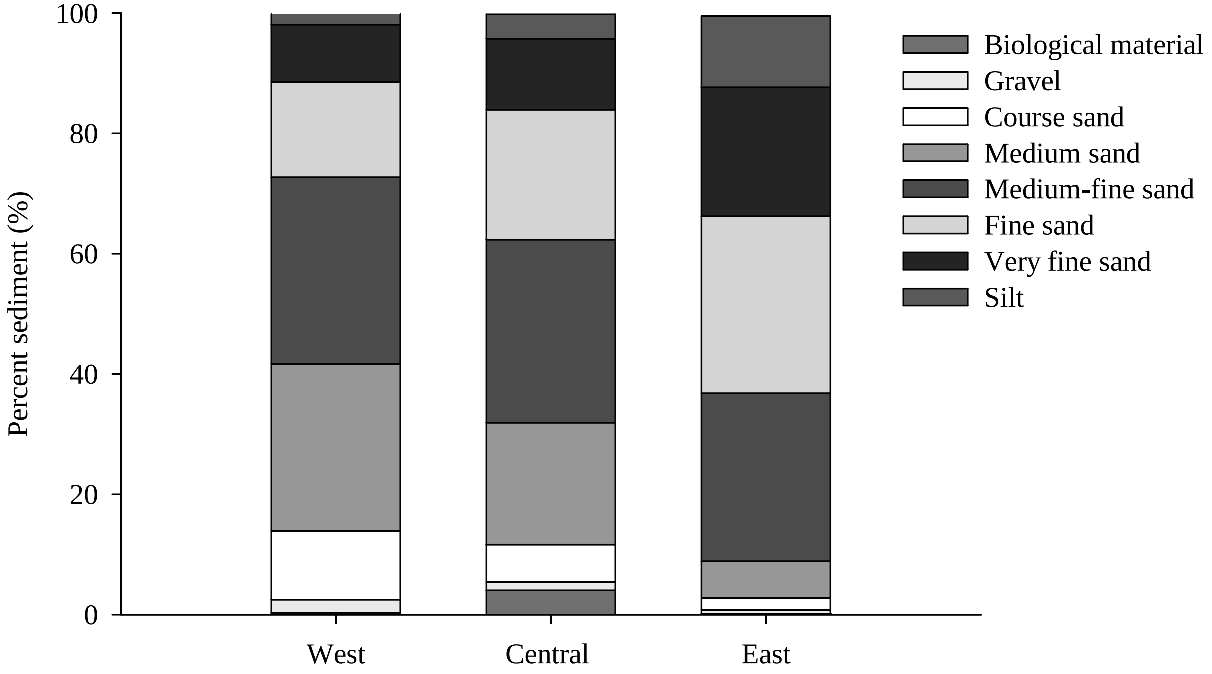


Figure 11. Percent sediment composition along a downstream gradient per area along Pamlico River, North Carolina from Fork Point Island, NC to the mouth of the Pungo River, NC sampled twice a month from August to November (2009-2010).

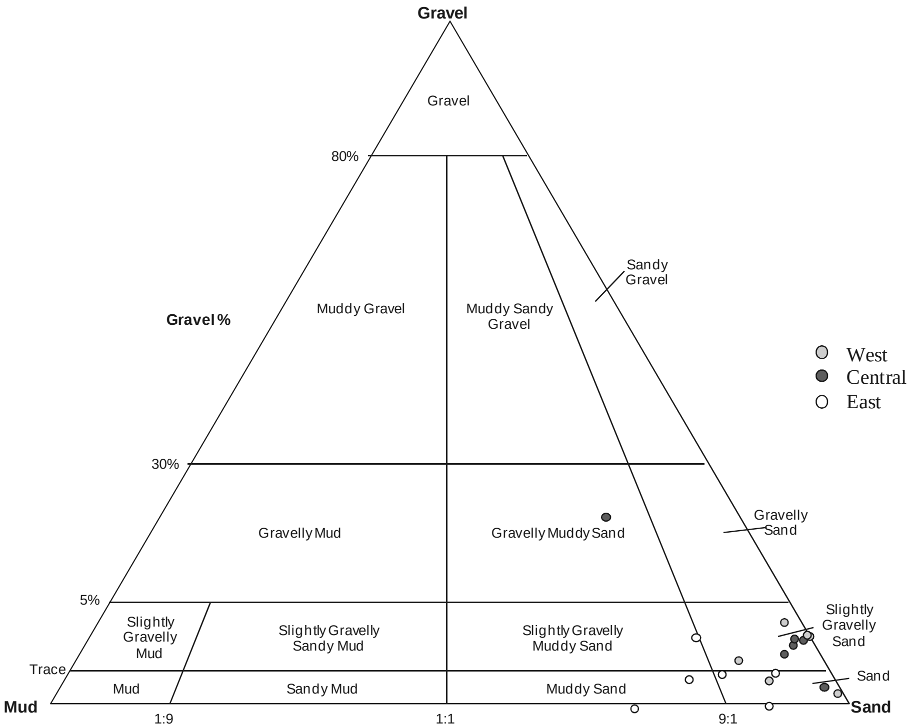


Figure 12. Sediment pyramid of sites sampled along Pamlico River, North Carolina in relation to practical size from Fork Point Island, NC to the mouth of the Pungo River, NC sampled twice a month from August to November (2009-2010).

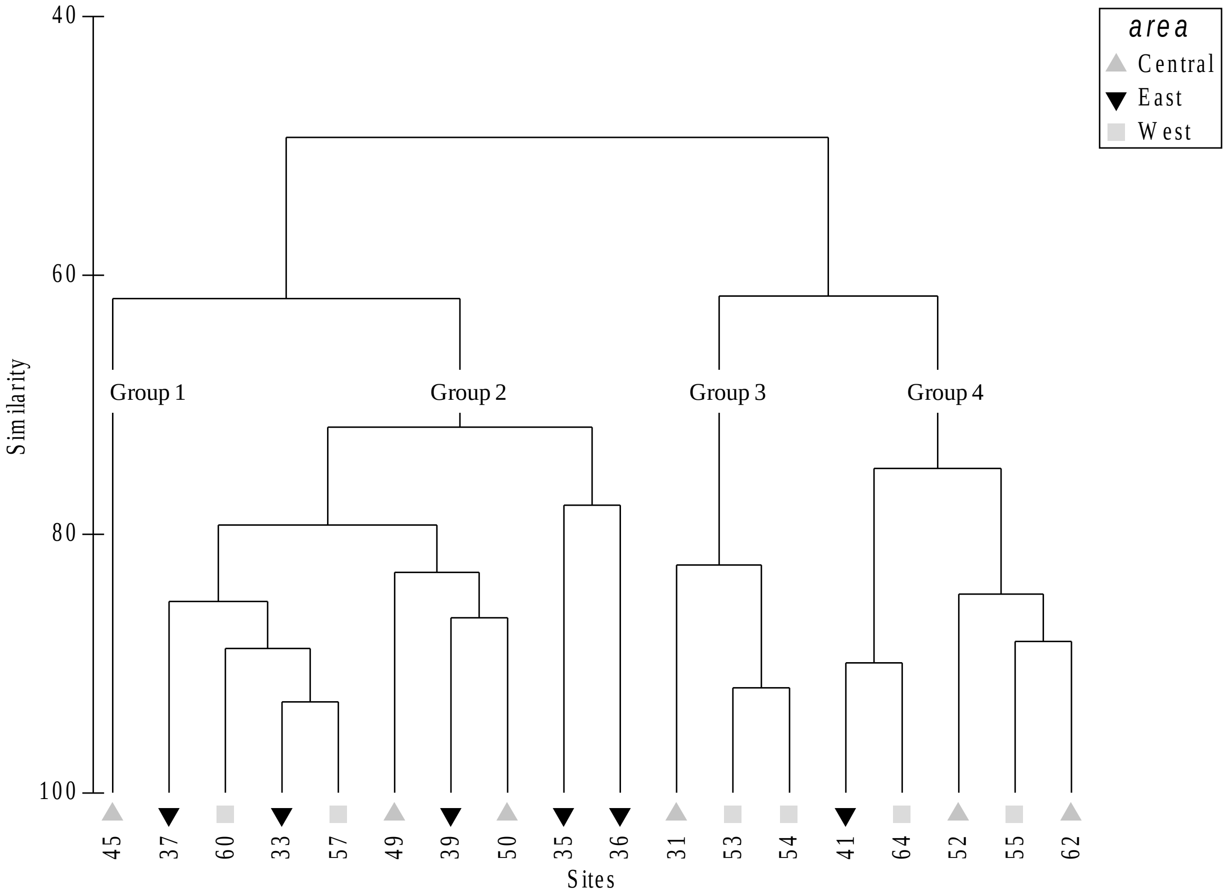


Figure 13. CLUSTER analysis of sediment type composition sampled along Pamlico River, North Carolina from Fork Point Island, NC to the mouth of the Pungo River, NC. With four groups represented and site 45 (Group 1) located in the Central area having low levels of similarity (<58%) when compared to all other sites (>50%).

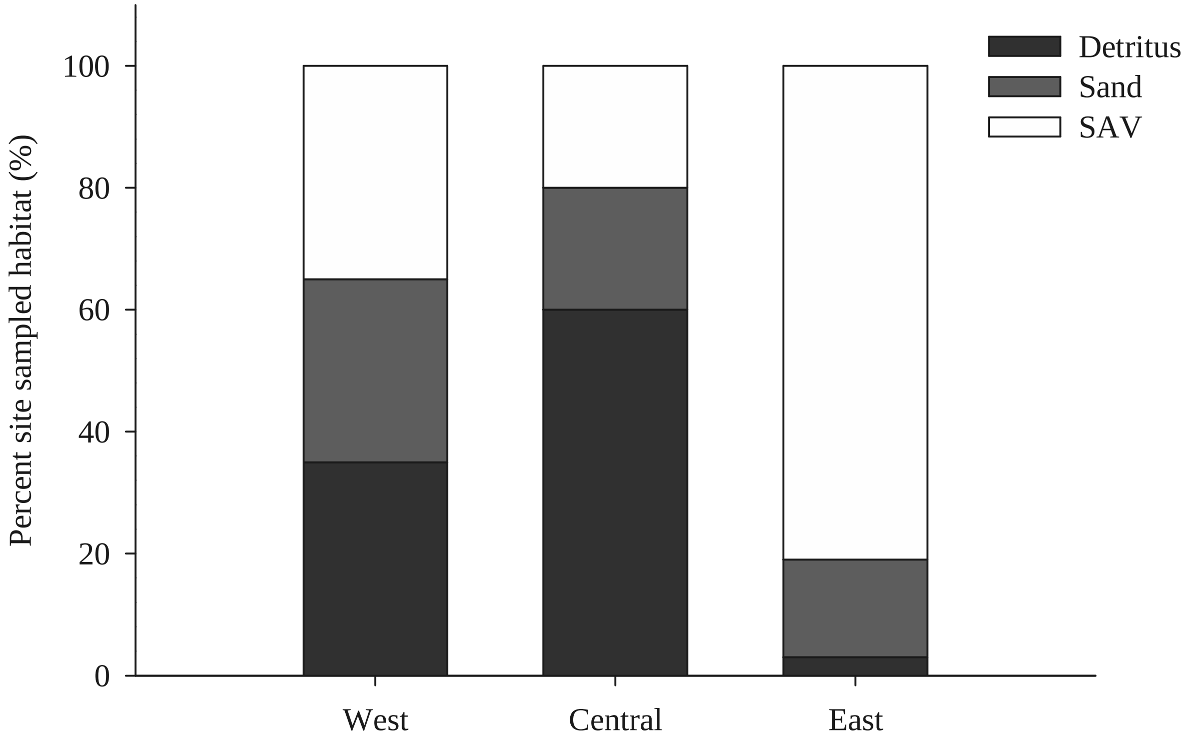


Figure 14. Percent of sampling sites classified as (detritus, sand, and SAV) in Pamlico River from Fork Point Island, NC to the mouth of the Pungo River, NC sampled twice a month from August to November (2009-2010) and using an 18-m long bag seine in Pamlico River, North Carolina.

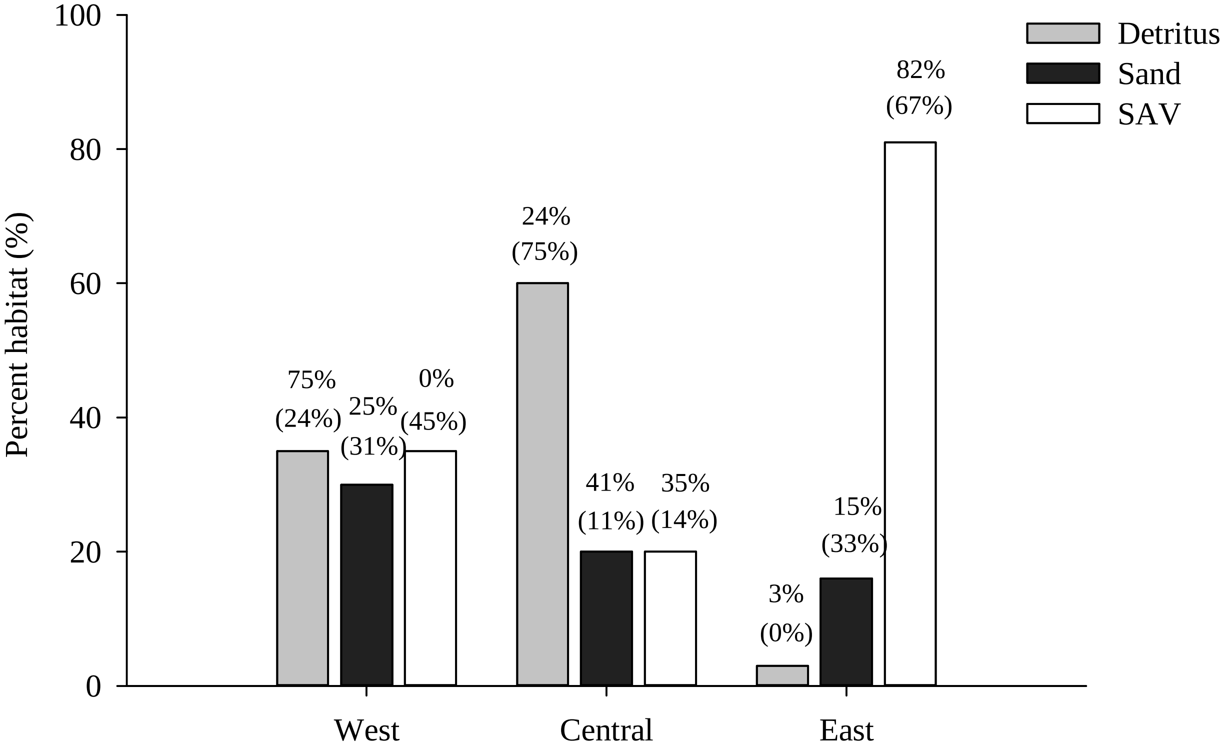


Figure 15. Percent of sampling sites classified as (detritus, sand, SAV) and percent spotted seatrout and red drum ( ) caught in each habitat along Pamlico River from Fork Point Isalnd, NC to the mouth of the Pungo River, NC sampled twice a month from August to November (2009-2010) and using an 18-m long bag seine.

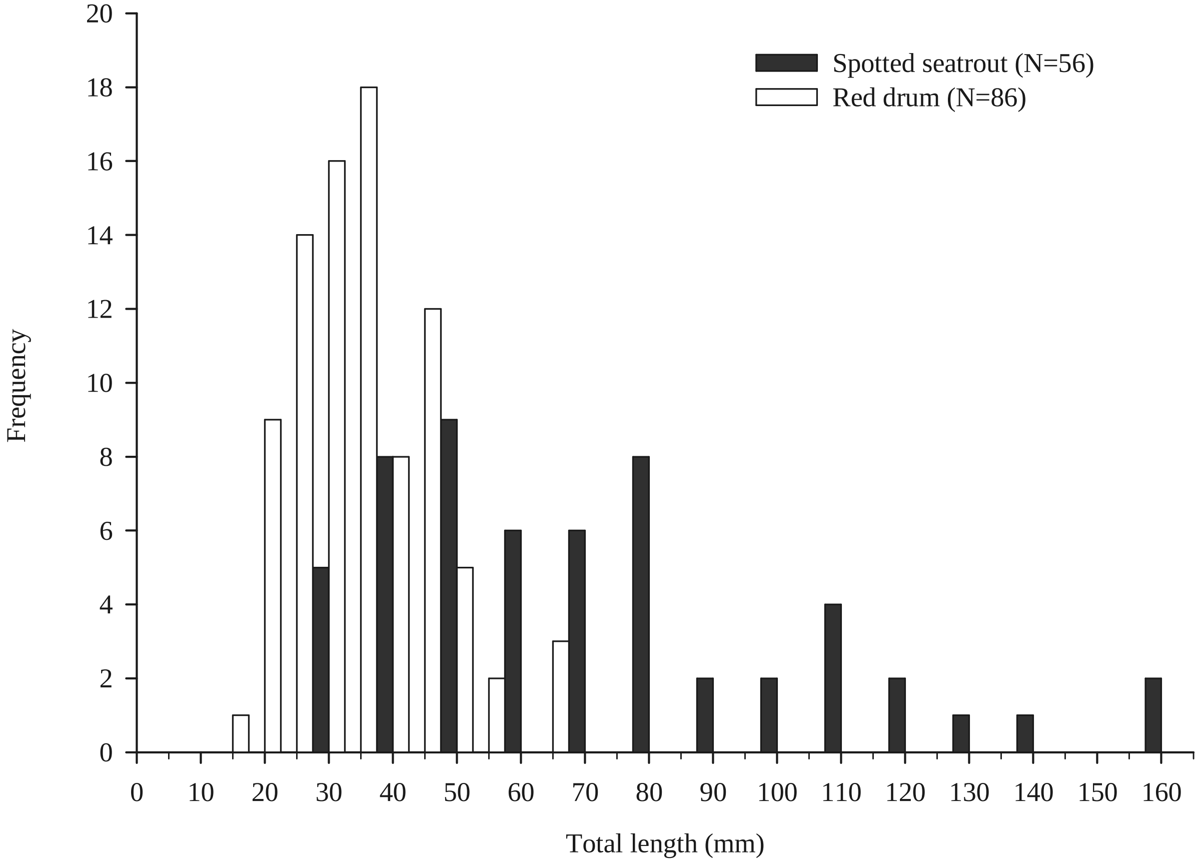


Figure 16. Length frequency distribution of juvenile spotted seatrout and red drum from August through November (2009-2010) along Pamlico River, North Carolina. Juvenile spotted seatrout lengths caught from 2009-2010 ranged from 30 mm to 160 mm with a mean (66.5± 4.4 mm). Juvenile red drum lengths ranged from 15 to 65mm with a mean (32.6 ± 1.1 mm).

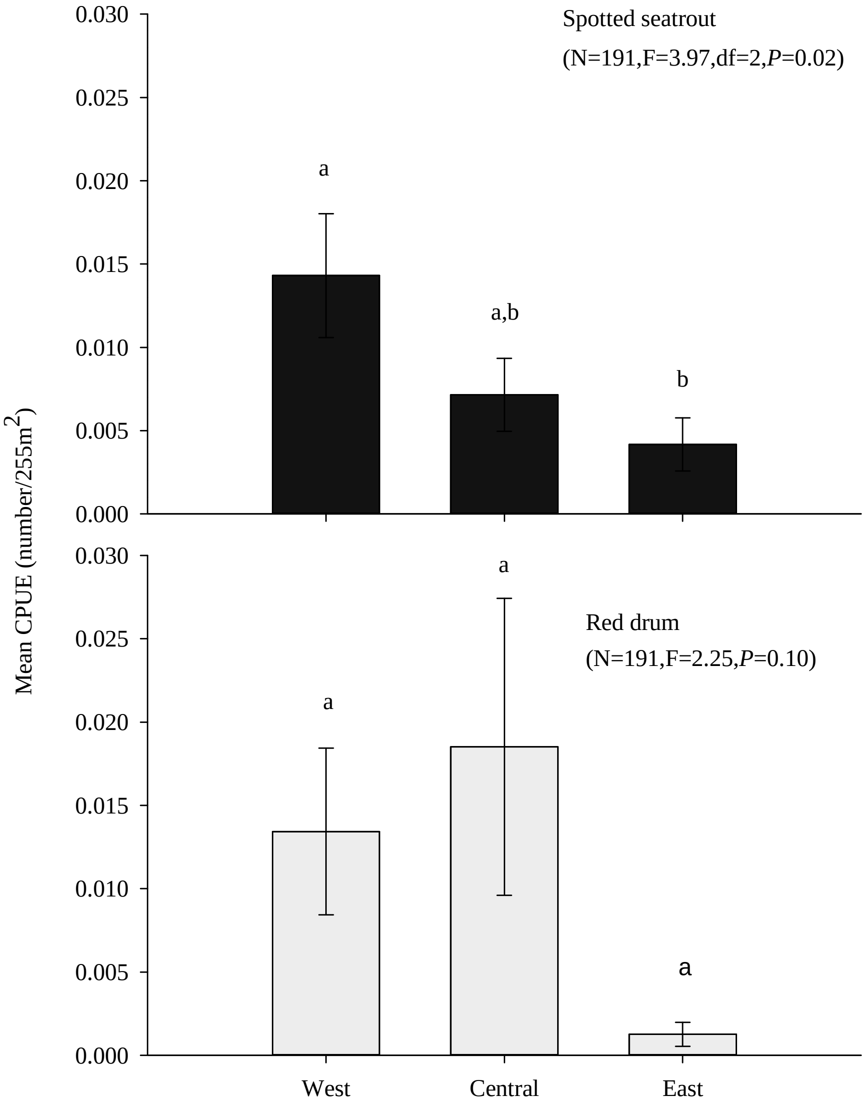


Figure 17. Mean catch per unit effort (CPUE) of juvenile spotted (black) seatrout and red drum (gray) per area (±1SE). Fishes were collected and all areas where sampled each time along sandy beach habitats from Fork Point Island, NC to the mouth of the Pungo River, NC twice a month from August to November (2009-2010) using an 18-m long bag seine in Pamlico River, North Carolina.

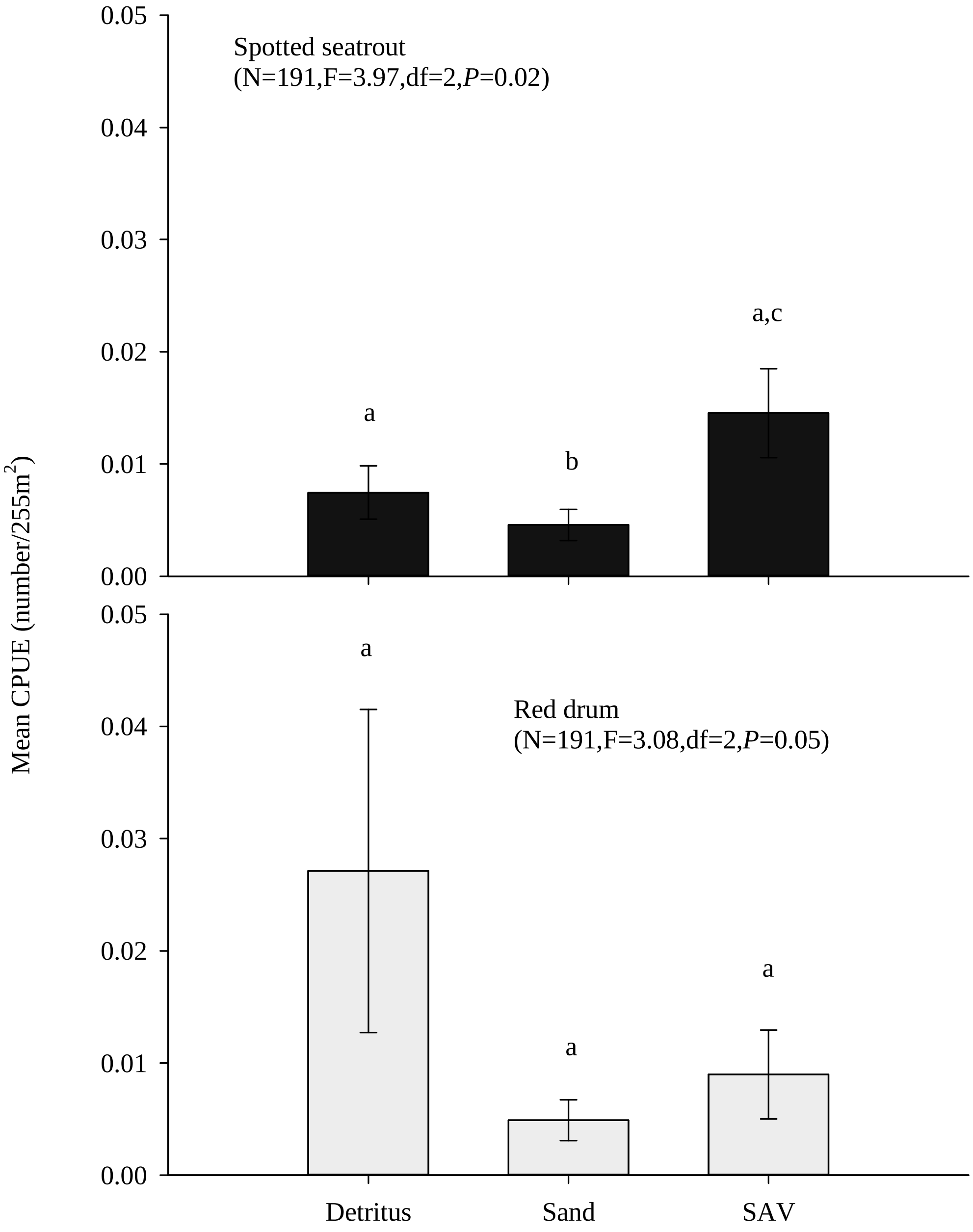


Figure 18. Mean catch per unit effort (CPUE) of juvenile spotted seatrout (black) and red drum (gray) per habitat type (±1SE). Fishes were collected and all areas where sampled each time along sandy beach habitats from Fork Point Island, NC to the mouth of the Pungo River, NC twice a month from August to November (2009-2010) using an 18-m long bag seine in Pamlico River, North Carolina.

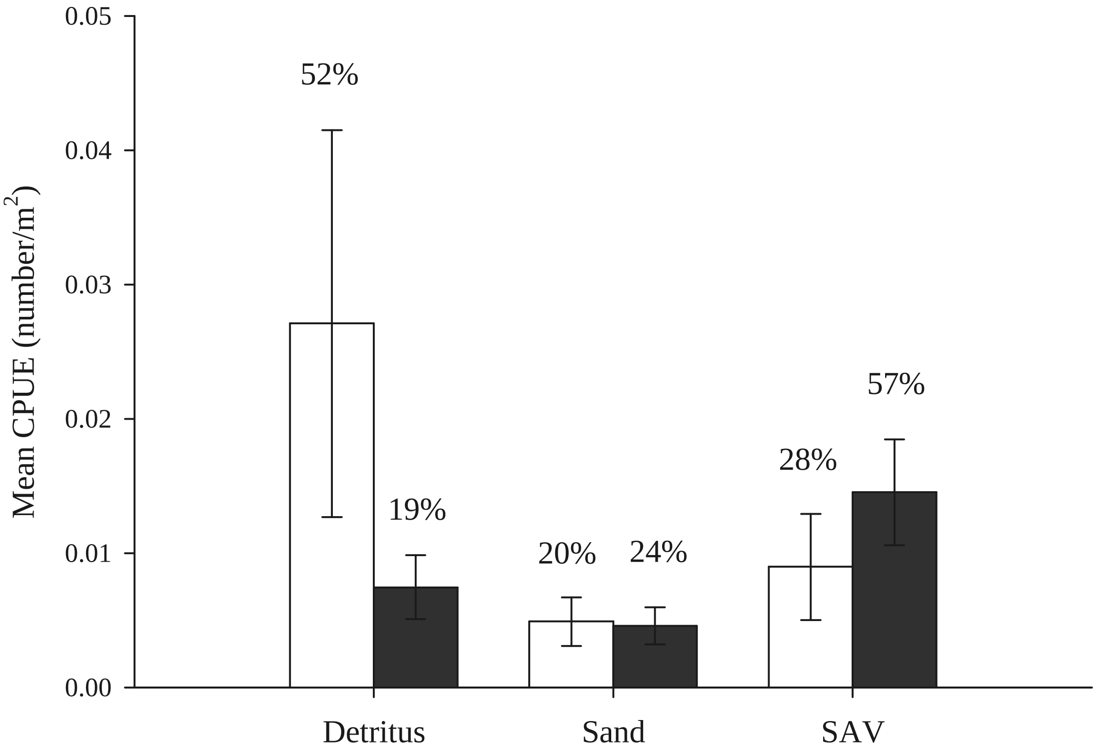


Figure 19. Catch per unit effort (CPUE) of juvenile spotted seatrout (black) and red drum (white) per habitat type (±1SE). Fishes were collected and all areas where sampled each time along sandy beach habitats from Fork Point Island, NC to the mouth of the Pungo River, NC twice a month from August to November (2009-2010) using an 18-m long bag seine in Pamlico River, North Carolina.

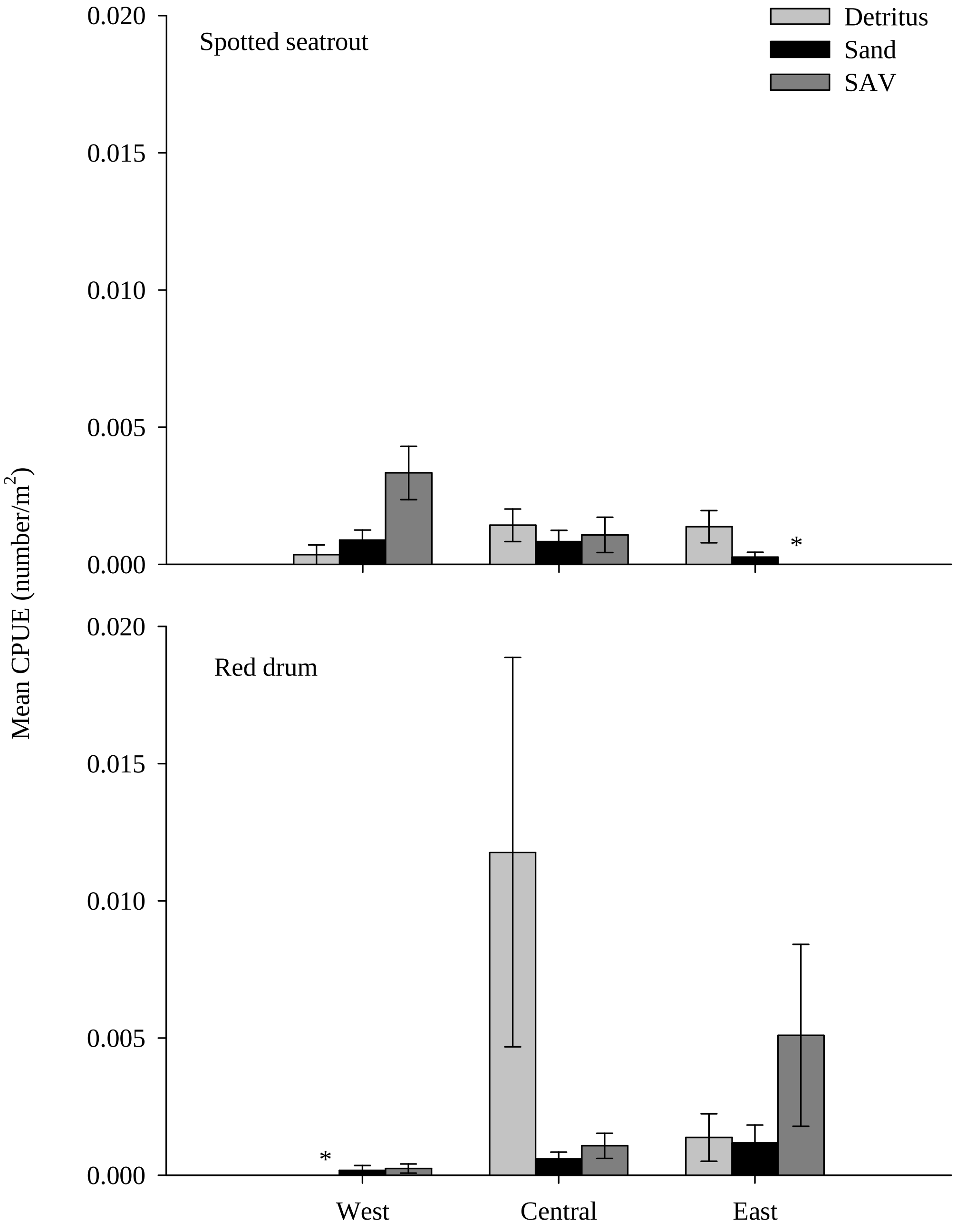


Figure 20. Mean catch per unit effort (CPUE) of juvenile spotted seatrout (top) and red drum (bottom) per habitat type (±1SE). Fishes were collected and all areas where sampled each time along sandy beach habitats from Fork Point Island, NC to the mouth of the Pungo River, NC twice a month from August to November (2009-2010) using an 18-m long bag seine in Pamlico River, North Carolina.

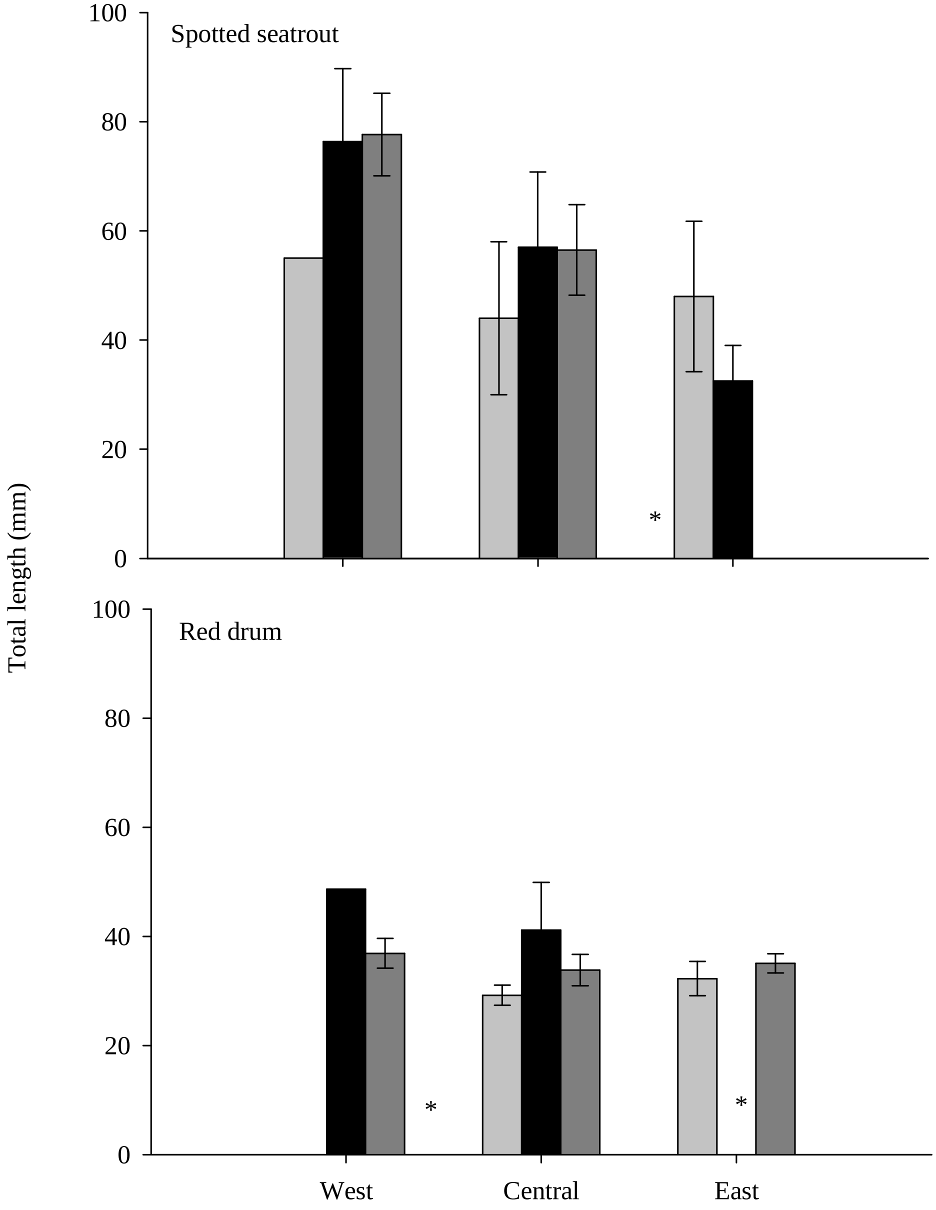


Figure 21. Total length (mm) of juvenile spotted seatrout (top) and red drum (bottom) among area (±1SE). Larger juvenile spotted seatrout and red drum occurring in the West area. Fishes were collected and all areas where sampled each time along sandy beach habitats from Fork Point Island, NC to the mouth of the Pungo River, NC twice a month from August to November (2009-2010) using an 18-m long bag seine in Pamlico River, North Carolina.

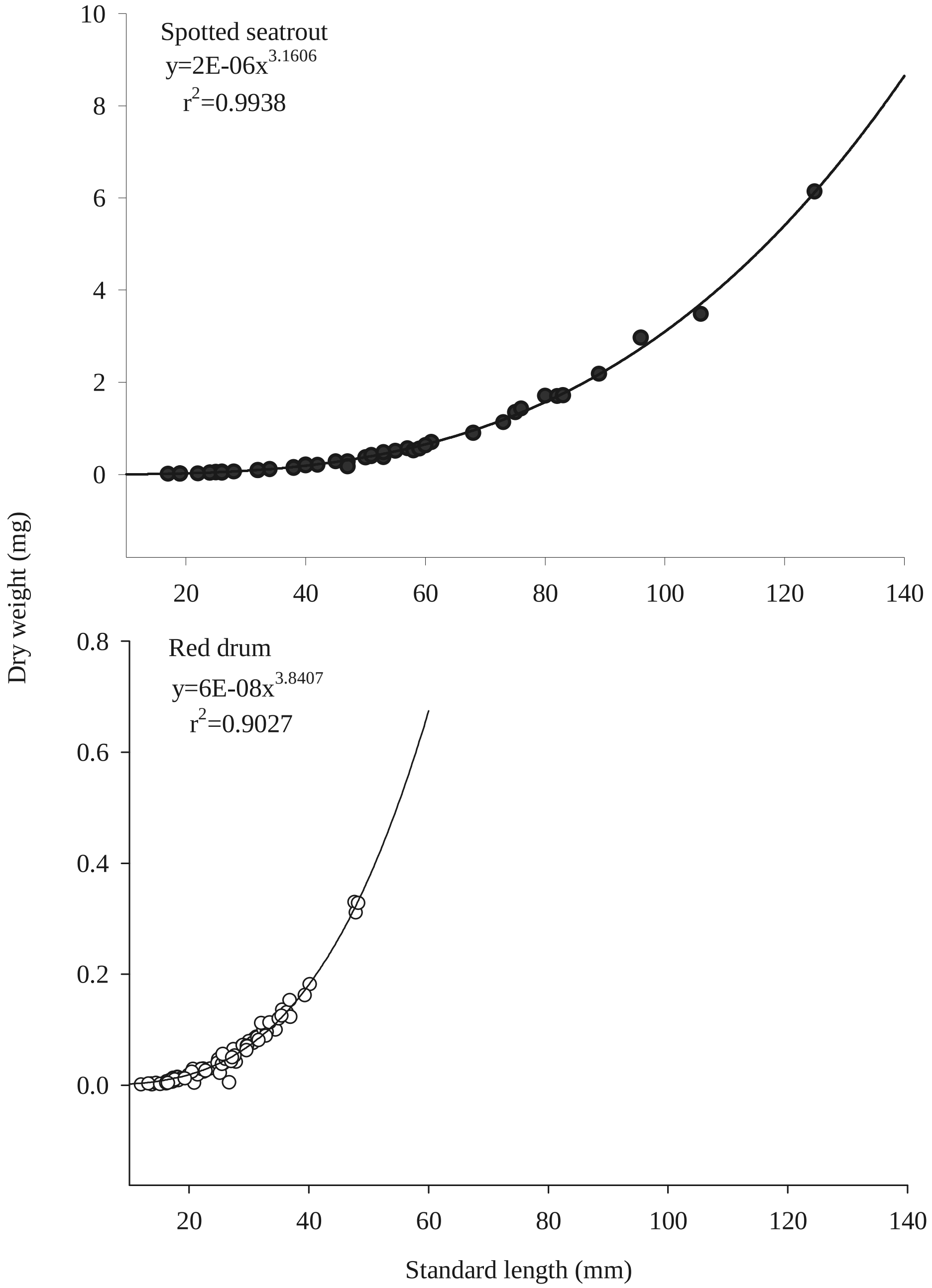


Figure 22. Juvenile spotted seatrout (black) and red drum (white) dry-weight (g) to length relationship. Standard length (mm) plotted on dry-weight (g) with power regression line overlaid. Fishes were collected and all areas where sampled each time along sandy beach habitats from Fork Point Island, NC to the mouth of the Pungo River, NC twice a month from August to November (2009-2010) using an 18-m long bag seine in Pamlico River, North Carolina.

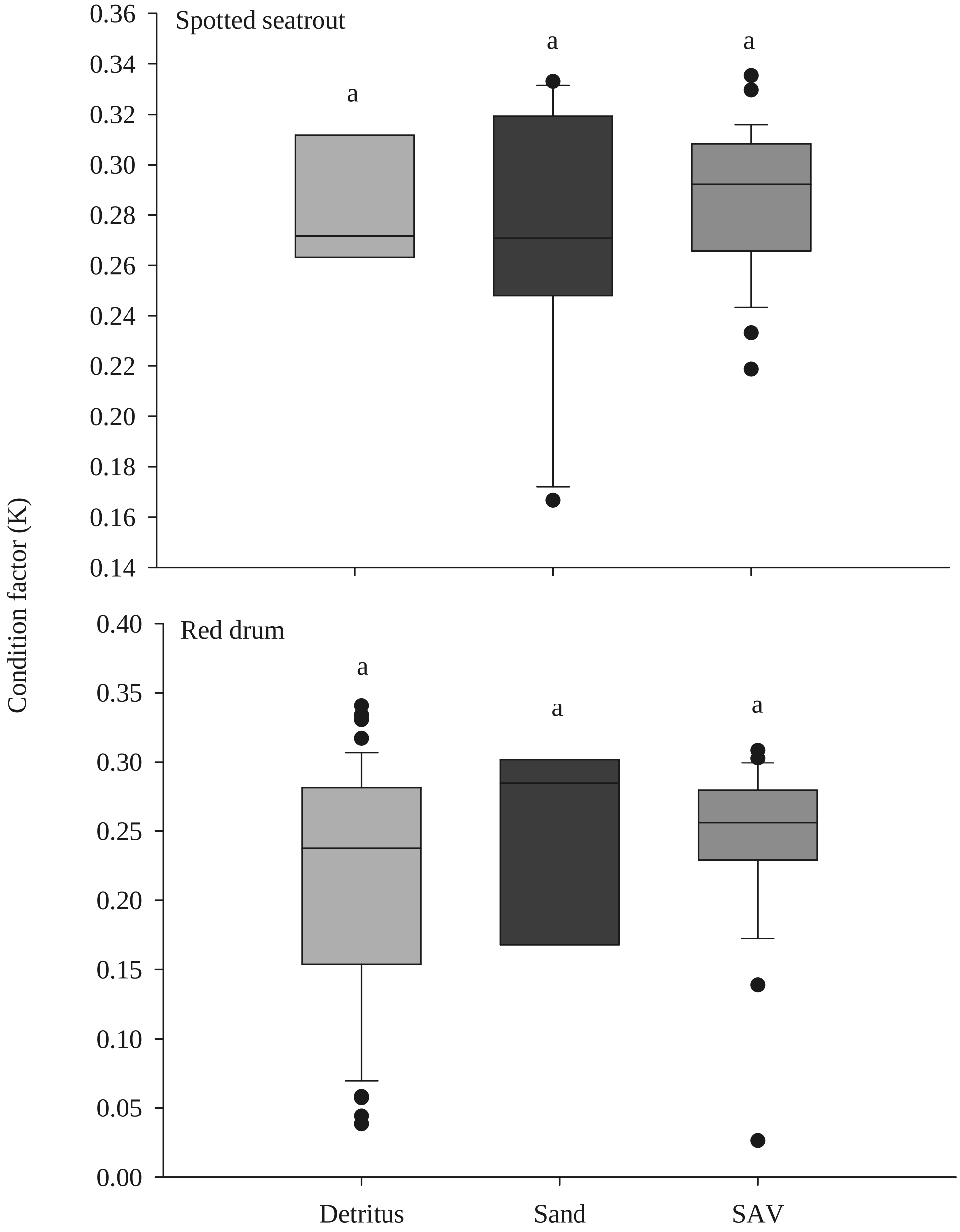


Figure 23. Fulton's Condition Factor (FCF) of juvenile spotted seatrout in (detritus, sand, and SAV) relation to habitat along Pamlico River, North Carolina (±1SE). Fishes were collected and all areas where sampled each time along sandy beach habitats from Fork Point Island, NC to the mouth of the Pungo River, NC twice a month from August to November (2009-2010) using an 18-m long bag seine in Pamlico River, North Carolina. The dots represent outliers and the whiskers represent the minimum and maximum of 100% of the values.

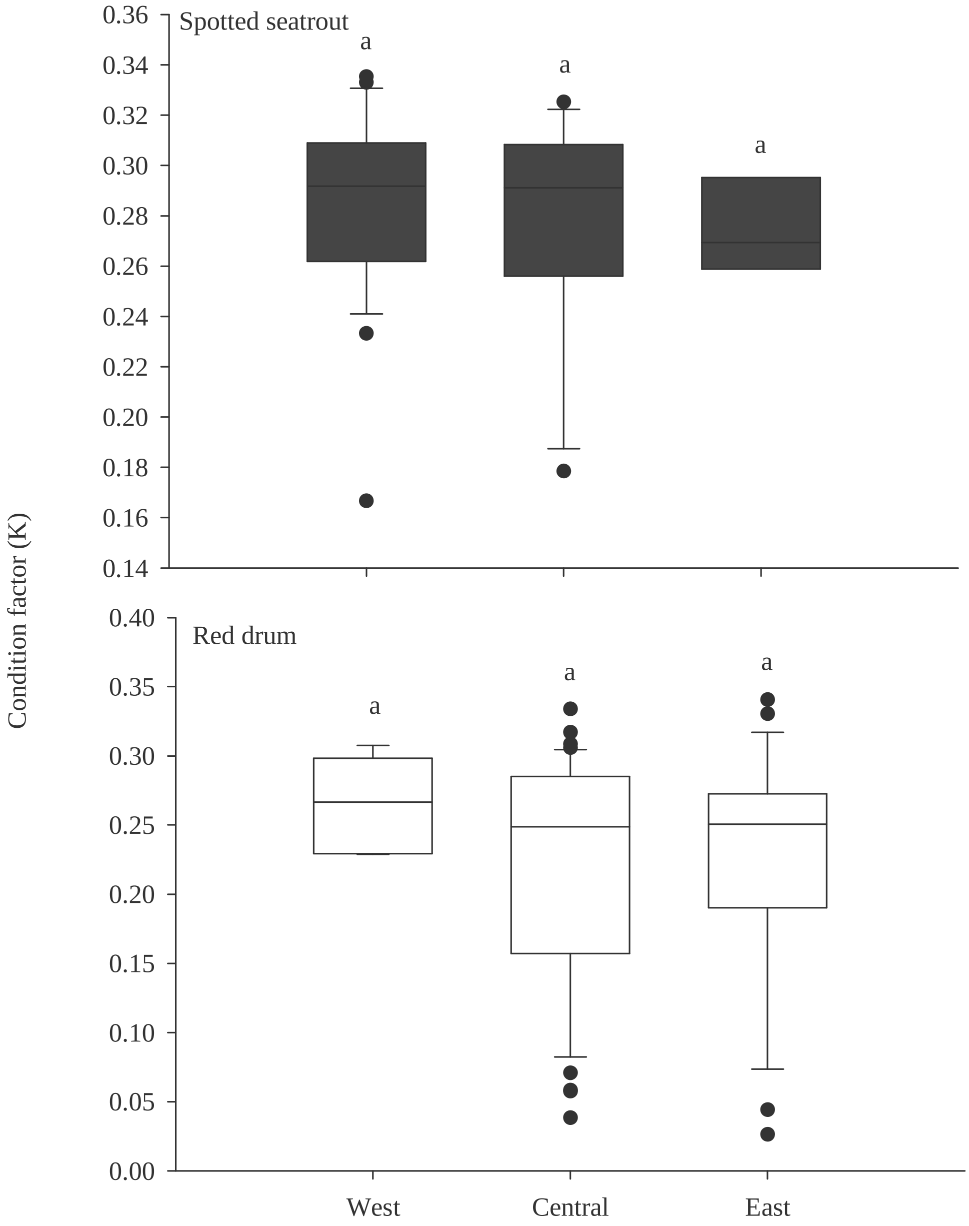


Figure 24. Fulton's Condition Factor (FCF) of juvenile spotted seatrout (black) and red drum (white) in (detritus, sand, and SAV) relation to area along Pamlico River, North Carolina (±1SE). Fishes were collected and all areas where sampled each time along sandy beach habitats from Fork Point Island, NC to the mouth of the Pungo River, NC twice a month from August to November (2009-2010) using an 18-m long bag seine in Pamlico River, North Carolina. The dots represent outliers and the whiskers represent the minimum and maximum of 100% of the values.

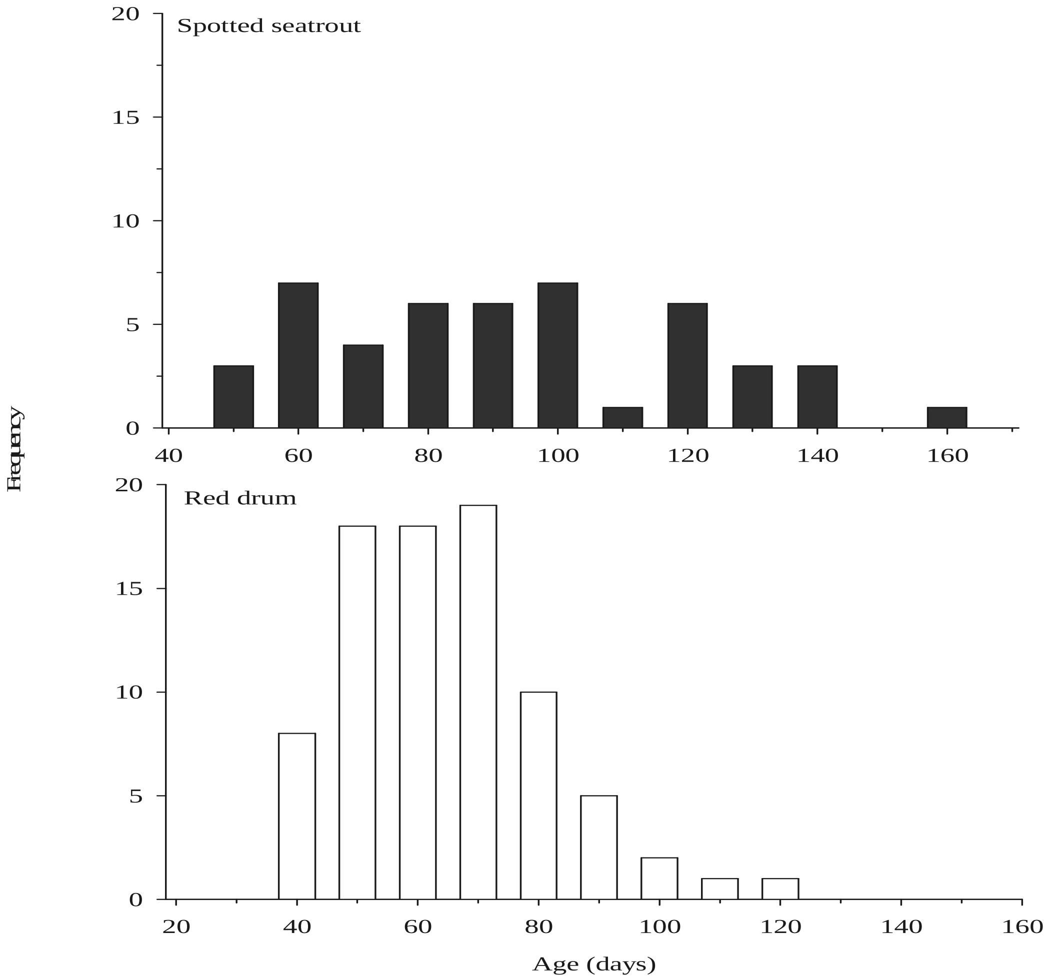


Figure 25. Age frequency of juvenile spotted seatrout (black) and red drum (white) along Pamlico River, North Carolina. Juvenile spotted seatrout lengths caught from 2009-2010 ranged from 40 to 120-d with a mean (89.2± 4.0). Juvenile red drum lengths ranged from 50 160-d with a mean (60.1 ± 1.8). Fishes were collected and all areas where sampled each time along sandy beach habitats from Fork Point Island, NC to the mouth of the Pungo River, NC twice a month from August to November (2009-2010) using an 18-m long bag seine in Pamlico River, North Carolina.

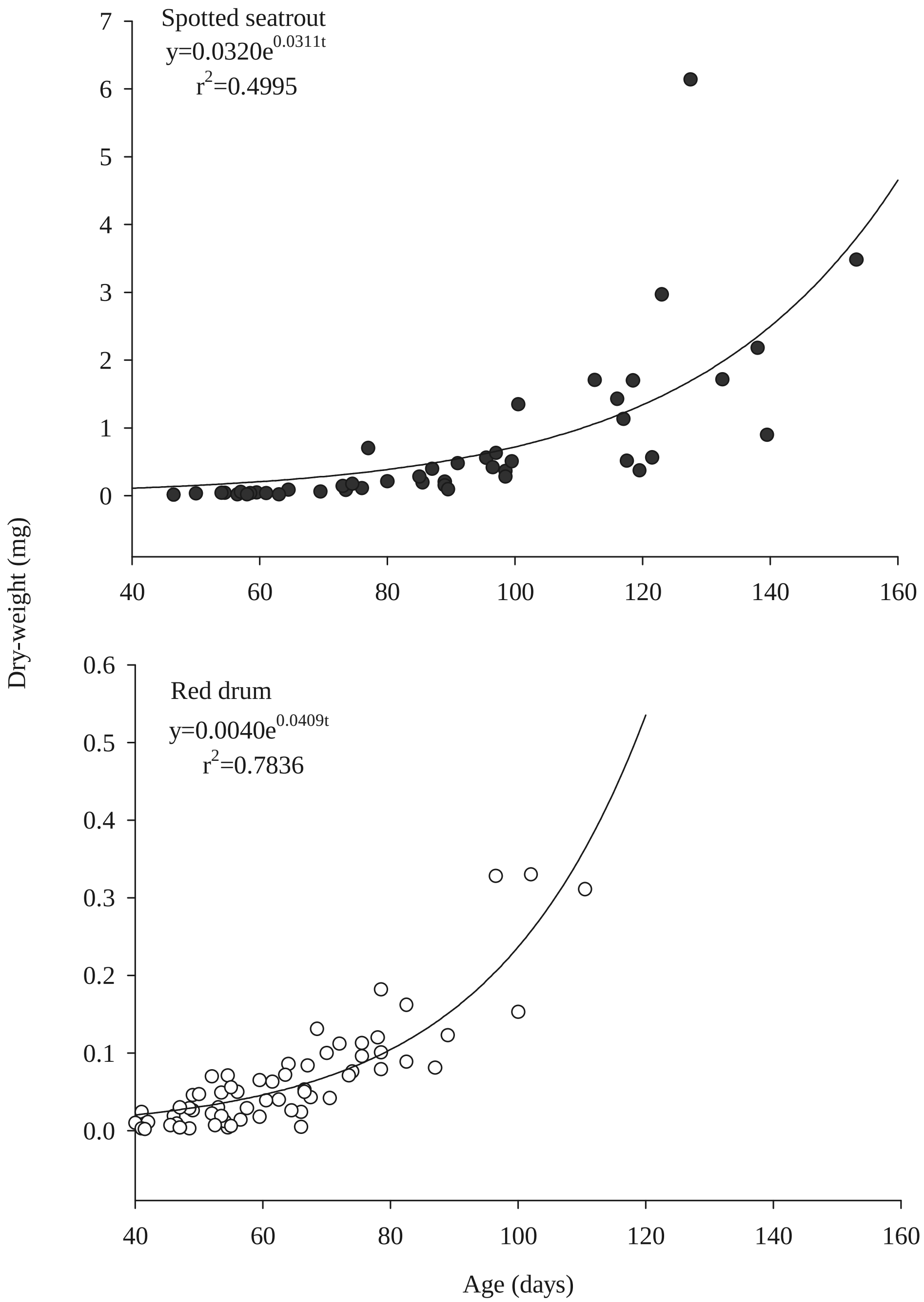


Figure 26. Juvenile spotted seatrout (black) and red drum (white) dry-weight (g) to age (days) relationship. Age (days) plotted on dry-weight (g) with exponential regression line overlaid. Fishes were collected and all areas where sampled each time along sandy beach habitats from Fork Point Island, NC to the mouth of the Pungo River, NC twice a month from August to November (2009-2010) using an 18-m long bag seine in Pamlico River, North Carolina.

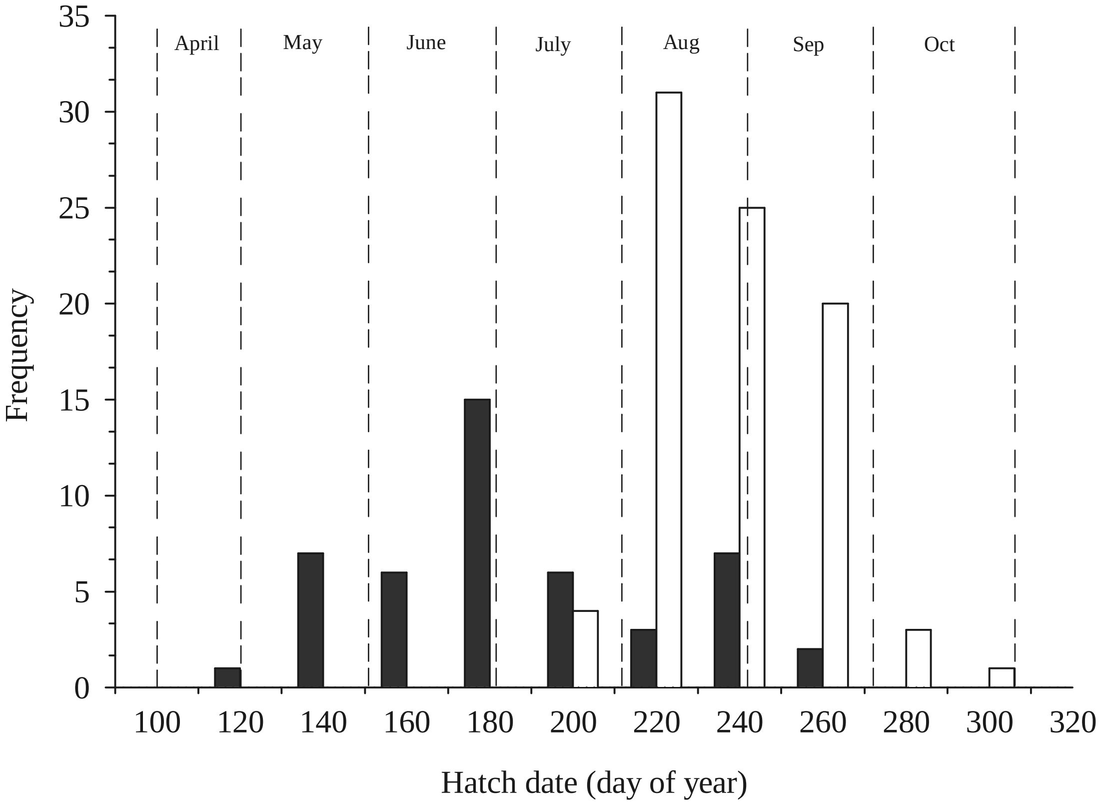


Figure 27. Hatch date of juvenile spotted seatrout (black) and red drum (white) per day of year, from August to November (2009-2010) using an 18-m long bag seine in Pamlico River, North Carolina. Fishes were collected and all areas where sampled each time along sandy beach habitats from Fork Point Island, NC to the mouth of the Pungo River, NC. Juvenile spotted seatrout and red drum peak hatching occurring in June and August, respectively.

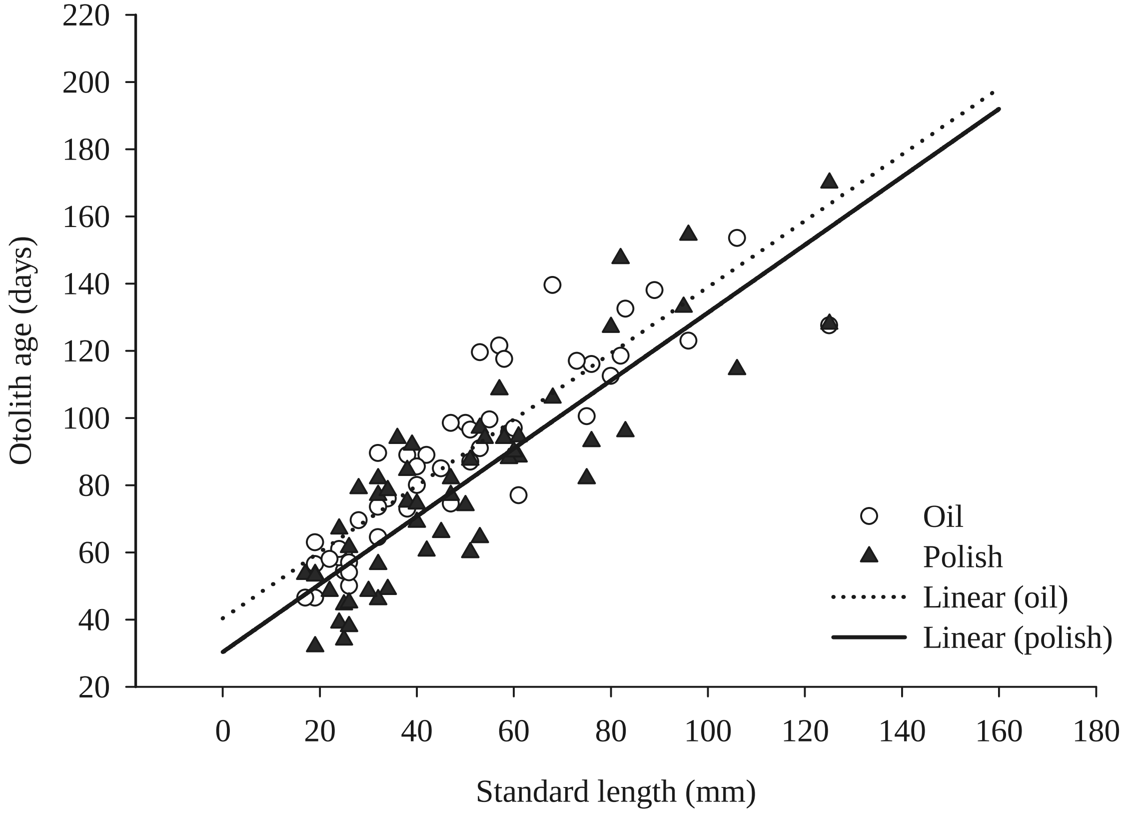


Figure 28. Juvenile spotted seatrout age to length relationship compare oil immersion and polishing techniques for ageing. Standard length (mm) plotted on age (days) with linear regression line overlaid. Oil immersion gives a higher estimate of age than the embedding method. Fishes were collected and all areas where sampled each time along sandy beach habitats from Fork Point Island, NC to the mouth of the Pungo River, NC twice a month from August to November (2009-2010) using an 18-m long bag seine in Pamlico River, North Carolina.

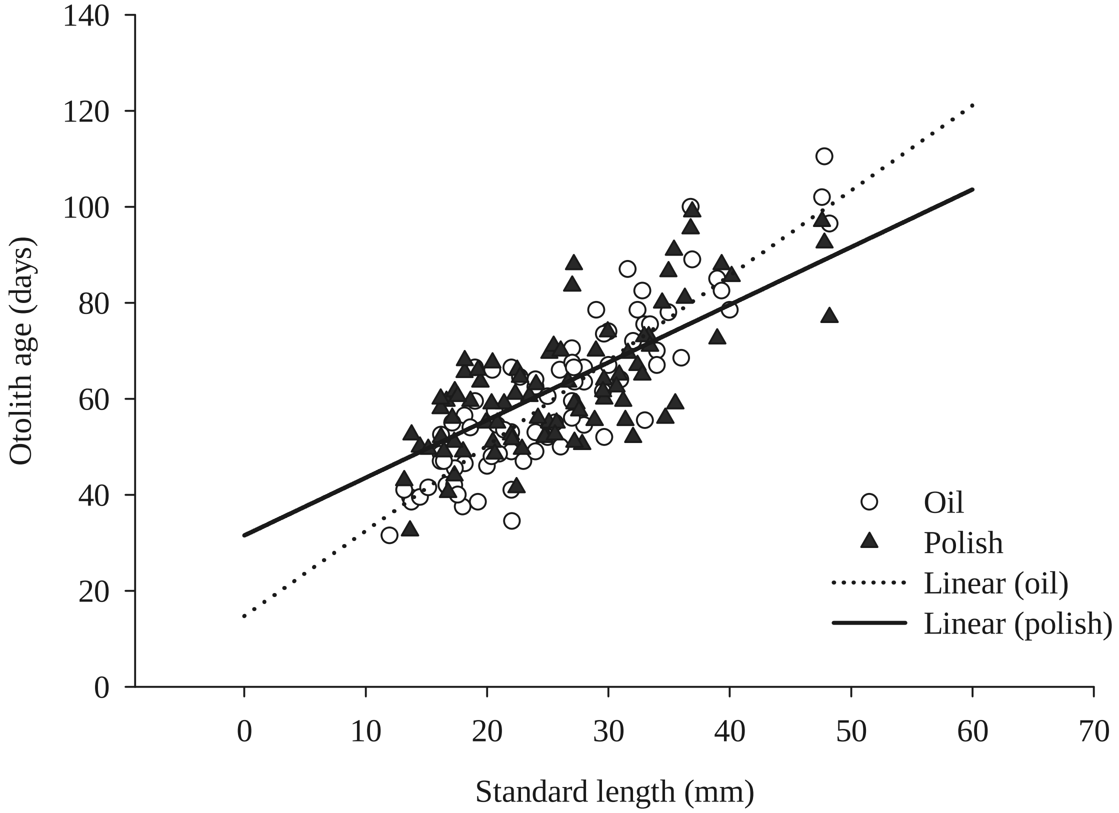


Figure 29. Juvenile red drum age to length relationship compare oil immersion and polishing techniques for ageing. Standard length (mm) plotted on age (days) with linear regression line overlaid. The oil immersion gives a lower estimate for fish <30 mm TL. However, the embedding method gives a higher estimate for fish >30 mm TL. Fishes were collected and all areas where sampled each time along sandy beach habitats from Fork Point Island, NC to the mouth of the Pungo River, NC twice a month from August to November (2009-2010) using an 18-m long bag seine in Pamlico River, North Carolina.

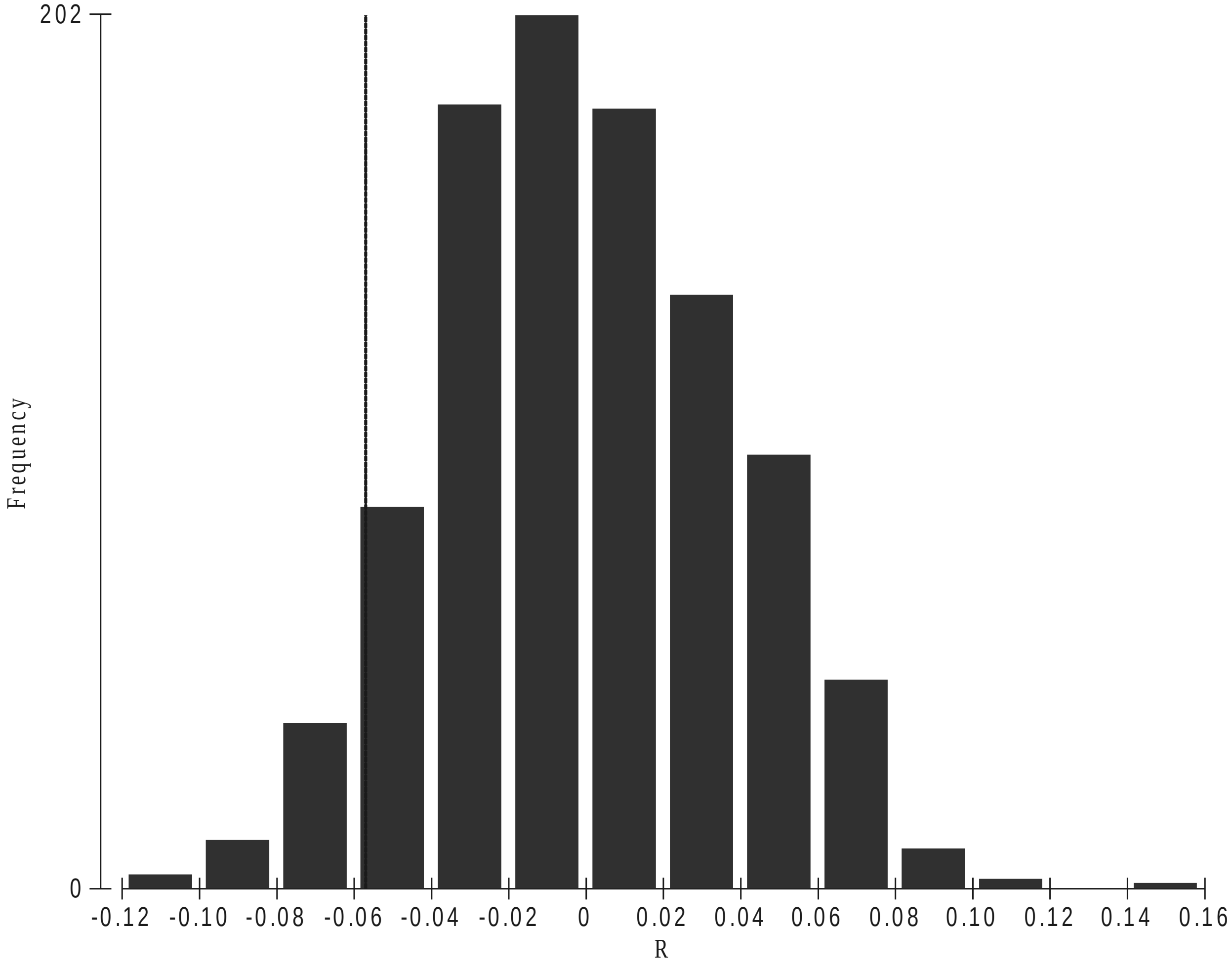


Figure 30. ANOSIM comparisons between the presences or absence of juvenile spotted seatrout and red drum along Pamlico River, North Carolina from Fork Point Island, NC to the mouth of the Pungo River, NC sampled twice a month from August to November (2009-2010) and using an 18-m long bag seine in Pamlico River, North Carolina. The one-way ANOSIM indicated that the presence or absence of both species had no affect on fish community structure (Global R=-0.058, p=94.2%).

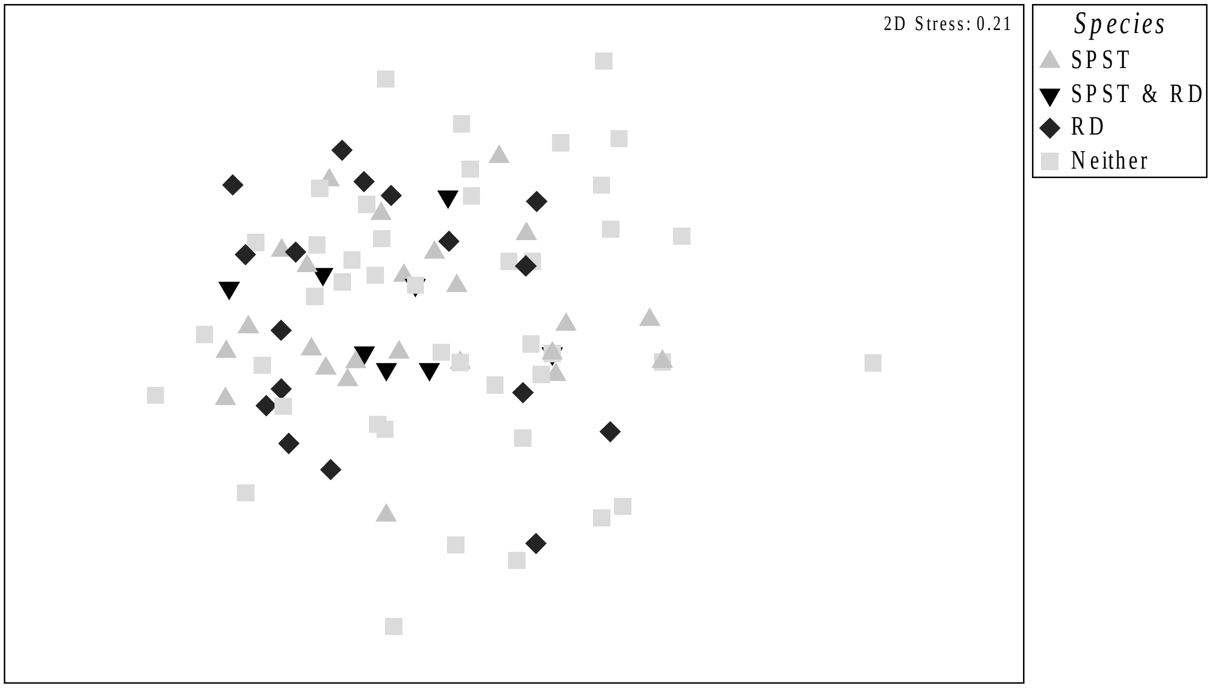


Figure 31. The non-metric multidimensional scaling (MDS) plot showing the presence or absence of juvenile spotted seatrout and red drum in relation to community structure along Pamlico River, North Carolina from Fork Point Island, NC to the mouth of the Pungo River, NC sampled twice a month from August to November (2009-2010) and using an 18-m long bag seine in Pamlico River, North Carolina.

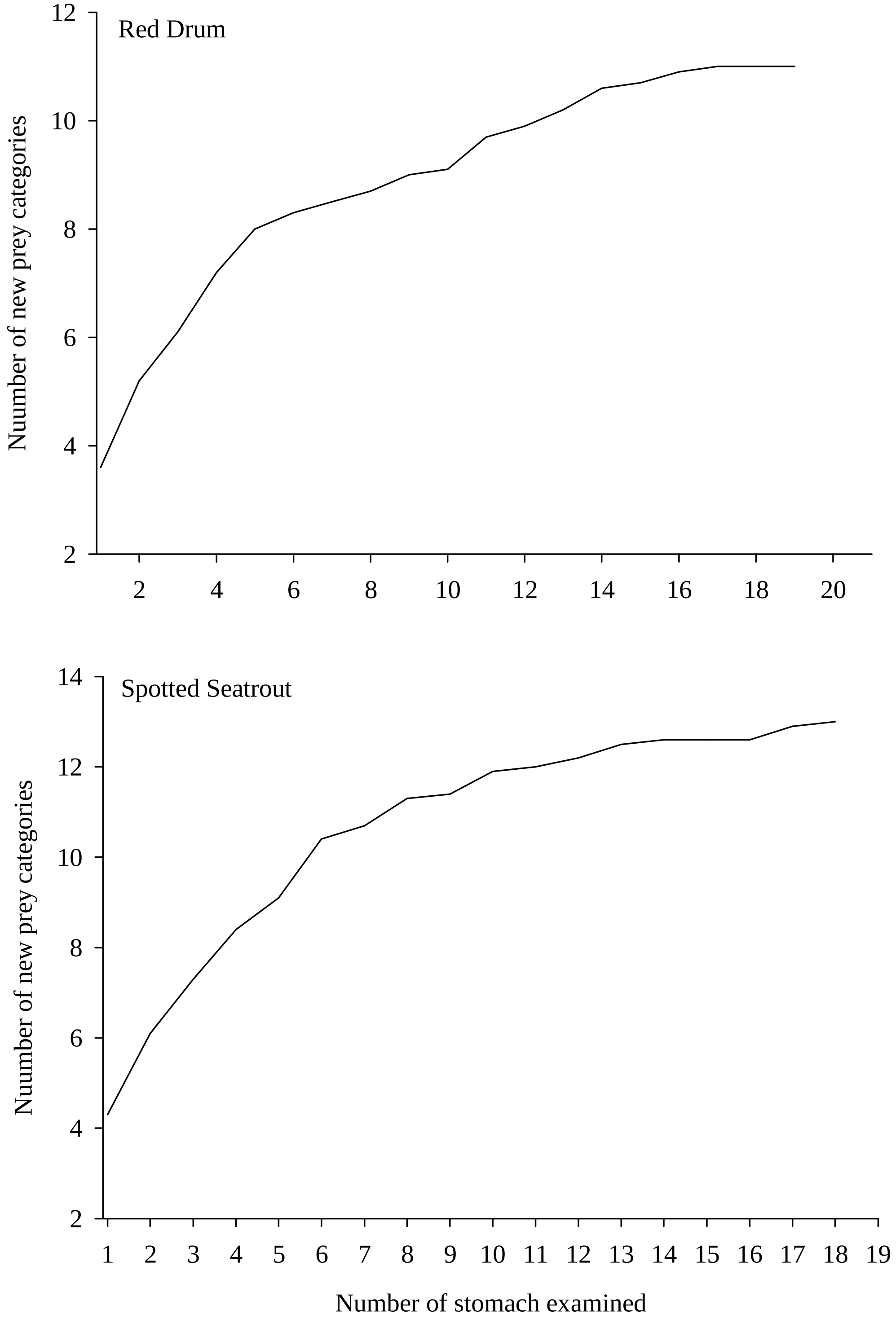


Figure 32. Cumulative prey curve for the total number of juvenile red drum and spotted seatrout. The black line is the mean number of new prey items calculated for each sample size after a 100-times randomization order of stomach contents.

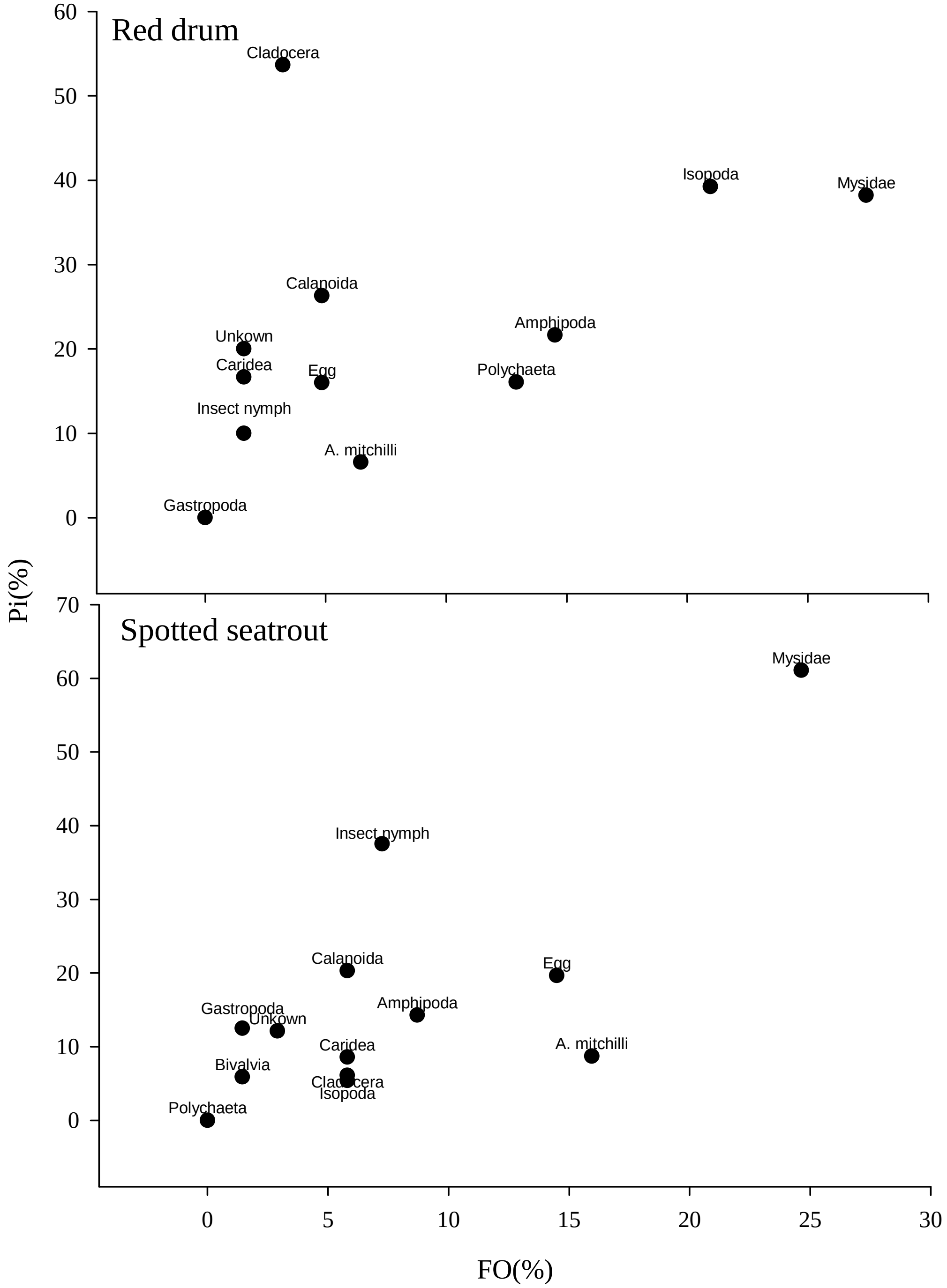


Figure 33. Relationship among prey specific abundance (%Pi) and frequency of occurrence (%FO) of food categories in juvenile red drum and spotted seatrout diet. Plots based on modified Costello graphical method (Amundsen et al. 1996).

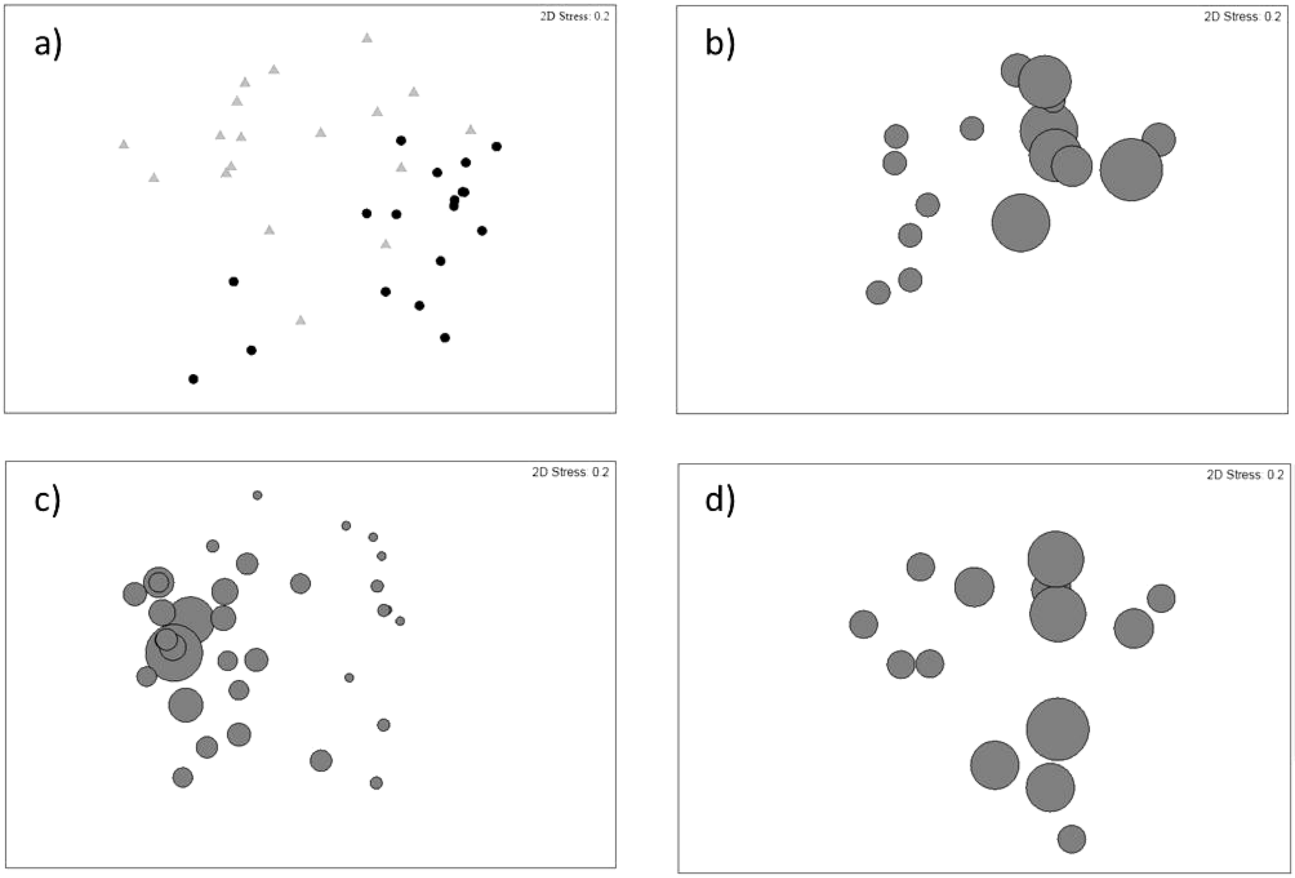
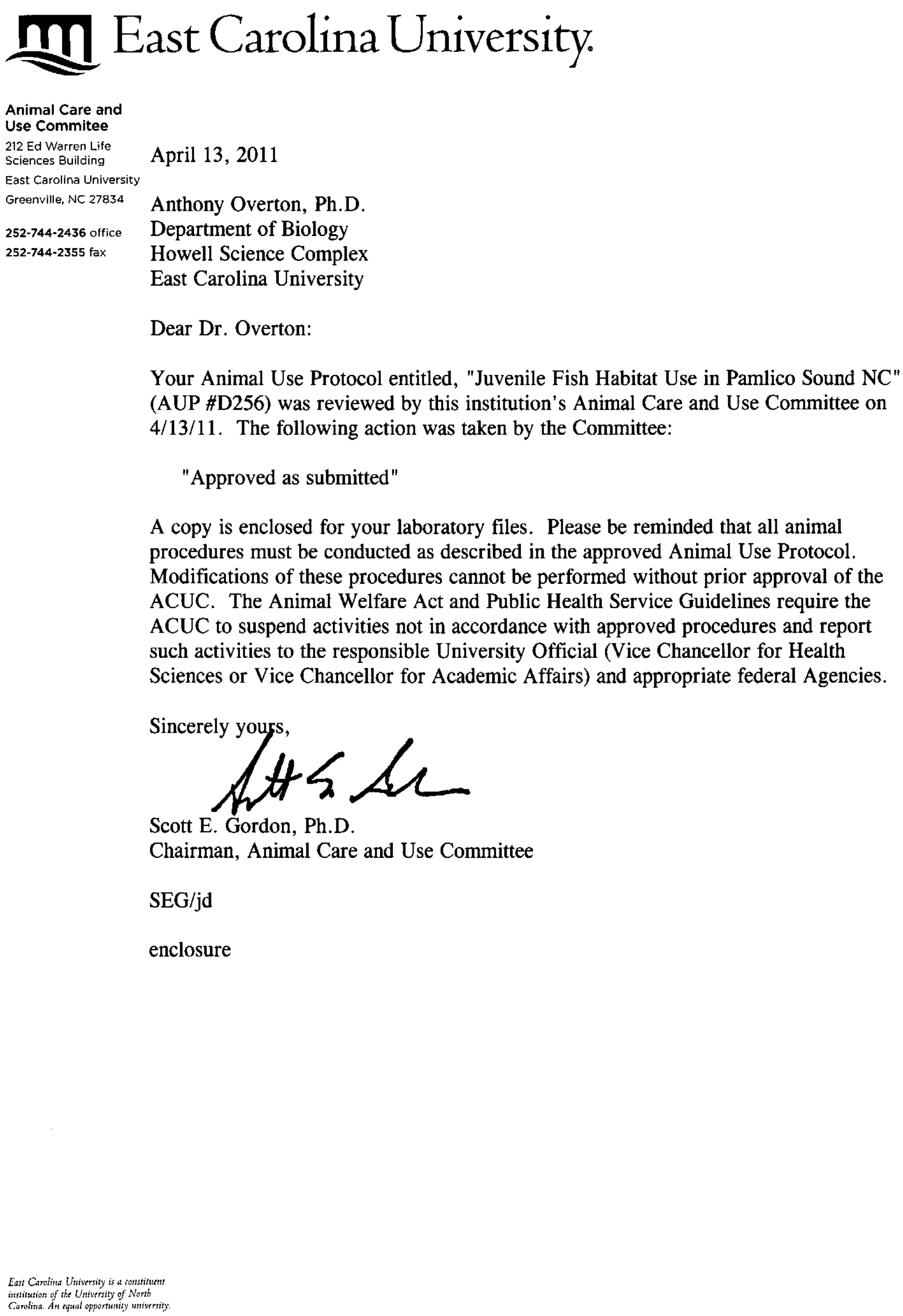


Figure 34. (a) Patterns in diet composition (%number) for juvenile red drum and spotted seatrout collected during August through November 2009-2010 in Pamlcio River, North Carolina demonstrated in a multidimensional scaling (NMDS) plot. Symbols that are close together have greater similarity than symbols that are further apart, bubble plots (b-d) are superimposed from the NMDS diet analysis. (a) Triangles represent spotted seatrout and closed circles represent red drum. Bubble size approximates relative proportion of a given taxa in the diet: (b) isopods, (c) mysids (d) amphipods.

APPENDIX A: ANIMAL USE PROTOCOL APPROVAL



East Carolina University.

Animal Care and Use Commitee
212 Ed Warren Life Sciences Building
East Carolina University
Greenville, NC 27834
252-744-2436 office
252-744-2355 fax

April 13, 2011

Anthony Overton, Ph.D.
Department of Biology
Howell Science Complex
East Carolina University

Dear Dr. Overton:

Your Animal Use Protocol entitled, "Juvenile Fish Habitat Use in Pamlico Sound NC" (AUP #D256) was reviewed by this institution's Animal Care and Use Committee on 4/13/11. The following action was taken by the Committee:

"Approved as submitted"

A copy is enclosed for your laboratory files. Please be reminded that all animal procedures must be conducted as described in the approved Animal Use Protocol. Modifications of these procedures cannot be performed without prior approval of the ACUC. The Animal Welfare Act and Public Health Service Guidelines require the ACUC to suspend activities not in accordance with approved procedures and report such activities to the responsible University Official (Vice Chancellor for Health Sciences or Vice Chancellor for Academic Affairs) and appropriate federal Agencies.

Sincerely yours,

Scott E. Gordon, Ph.D.
Chairman, Animal Care and Use Committee

SEG/jd

enclosure

East Carolina University is a constituent institution of the University of North Carolina. An equal opportunity university.